

Consequences of the Bombing in Vietnam

THE Vietnam war as such is no part of *Nature's* parish (see *Nature*, **239**, 182; 1972) but the events of the past two weeks and the resumption by the United States of intensive bombing of North Vietnam above the 20th Parallel have raised issues which are bound to have an important influence, in the months ahead, in ways which the Administration in the United States has clearly either overlooked or deliberately ignored. The reasons why the bombing was resumed are, even now, by no means apparent. The United States Administration says that the breakdown of the peace negotiations in Paris a few weeks after President Nixon's successful re-election was a direct result of the intransigence of the North Vietnamese, but there is at least as much to be said for the view that the United States went back to Paris, after the election, with demands for a tightening of the draft agreement which could reasonably have been calculated to precipitate a breakdown. On the face of things, United States policy has been successful in that it has compelled a resumption of negotiations, with technical meetings in Paris this week and the arrival of Dr Henry Kissinger in Paris on Monday. By good luck, the Administration has been able to announce a resumption of the negotiations without having to acknowledge the force of the protests over Christmas that intensive bombing is, to say the least, an indecent substitute for diplomacy. What the United States has neglected, in its calculations of the consequences of the bombing, is that those governments which have protested will now be anxious to ensure that North Vietnam has a fair crack of the whip in Paris.

The coincidence of the bombing with the enlargement of the European Community is another part of the Administration's calculations which has either been done inaccurately or not done at all. Fortunately, the European Community has not become a kind of anti-American organization as might have been expected in the 1950s. Inevitably, however, especially now that it is a larger community than the United States itself, there is bound to be a re-examination of the extent to which Europe and the United States should coordinate their policies, not merely in defence but in economic affairs. And in the year ahead, there will be several opportunities for a redefinition of the transatlantic relationship. Where defence is concerned, the talks on Mutual Balanced Force Reductions scheduled for the spring and the European Security Conference in the summer will provide opportunities for European governments to work towards a lessened dependence on the United States. Although the United States itself will no doubt welcome an opportunity to reduce its military commitment to Europe, the Administration may well be discomfited by the tendency there will be to exclude it from other matters, some of them lying at the heart of the arrangements for the deployment of strategic power in Europe. But this year will also see another round of negotiations between Europe and the United States in Paris, the long awaited

resumption in GATT of the Kennedy Round of tariff reductions, still incompletely implemented. There is now a serious danger that the bombing of North Vietnam will incline European governments against the further liberalization of trade across the Atlantic and with developing countries which has for several years been urgently necessary. It is to be hoped that in both these important areas, the governments of Europe will restrain themselves. Mr Nixon will not, after all, be President indefinitely, while the enlargement of the European Community and the diminution of the stature of the United States typified by the irrationality of the bombing over Christmas will make it easier for Europe to talk as an equal to the United States.

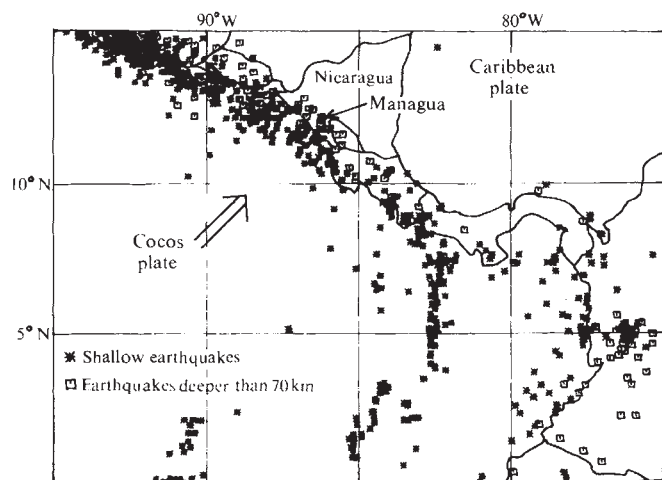
Another question raised by the bombing concerns the future of Dr Henry Kissinger, the architect of Mr Nixon's international policy in the past four years and the man chiefly responsible for the draft agreement on a peace settlement in Vietnam. Dr Kissinger is a distinguished scholar who deliberately took the risk of joining Mr Nixon's Administration in the hope, substantially justified by the experience of the past few years, that he would be able to exercise a liberalizing influence but no doubt conscious of the way in which many of his predecessors had been pilloried by academics for their association with the Administration's policy in Vietnam. There is now good reason to suspect that Dr Kissinger is not responsible for the bombing or even for the breakdown of the peace negotiations which preceded it. Indeed, there is everything to suggest that Dr Kissinger has been taken for a ride by the events of the past few weeks. In the circumstances, it is important that the academic community, and especially the students, in the United States should appreciate the difficulty and the delicacy of his position and that he should be given credit for his liberalizing influence in the past four years. But there are probably also good reasons why Dr Kissinger himself should now recognize that the time has come to return to Harvard, where his chair in international relations has been held vacant for the past four years but where a successor is likely to be appointed if he continues at the White House. Dr Kissinger's dilemma about the choice to make has been much publicized in recent weeks but the bombing should have helped make up his mind.

Nicaraguan Earthquake

THE awful lesson to be learnt from the earthquake which destroyed Managua, capital city of Nicaragua, on December 23, is that there may really be no economically feasible lesson in it at all. The earthquake killed upwards of 5,000 people and left Managua in ruins. The reason for it can be put all too precisely. The Cocos plate in the East Pacific is inexorably being thrust under Central America and the boundary between the Cocos and the Caribbean



plate is along the western shore line of the Central American isthmus. The process of overriding the 50 km thick Cocos plate and essentially of swallowing it at a rate of several centimetres a year produces a belt of high seismicity along this whole shoreline. Several hundred detectable earthquakes occur each year on this 2,500 kilometre convergence zone. The majority of them are underwater, but a significant fraction occur on land. The Managua earthquake was a magnitude 6 event—a factor of a hundred smaller in fault length than the magnitude 8 events such as San Francisco 1906 and Alaska 1964. The destruction wreaked by smaller events depends on close proximity (perhaps to within a few kilometres) of civilization. Thus this earthquake will go down with Agadir 1960 and Skopje 1963 as events of magnitude 6 which occurred near centres of population and killed thousands.



Seismicity from 1961–9. Map is produced by U.S. Department of Commerce, National Earthquake Information Center.

Managua does not lack a history of seismic damage. In 1926, half the city was destroyed by an earthquake. In 1955 and 1964, it suffered further damage. There is not a shred of evidence to suggest that the most recent earthquake will do more than temporarily relieve built-up stresses. Past seismicity is rather a good guide to where future earthquakes can be expected. And in this present state of seismological understanding there seems no way of capitalizing on information gained during this earthquake without enormous expense. The four possible courses of action are to stop earthquakes, to predict them, to remove towns from earthquake zones and to enforce rigid building codes. The first course would involve either stopping the Earth's convective motion or lubricating all known faults. The second course looks slightly more plausible on the basis of recent Russian results—provided that the technology is available, a high false alarm rate is acceptable and people have time to move. The third course is somewhat more plausible, although in a country like Nicaragua this would involve permanently evacuating the whole western coastal areas—in California presumably the only utterly safe thing to do would be to evacuate San Francisco and Los Angeles. The fourth course has met with some signal success in the United States. The San Fernando earthquake (1971) provided striking evidence of the efficacy of building codes, but there is, of course, an urgent need for similar codes for highway bridges, transformers and the interior features of buildings. Whether even a rudimentary code could be enforced at

any reasonable price in Nicaragua or other countries in this region is doubtful, to say the least.

Decisions on whether a government should attempt an earthquake protection programme are clearly as much political as scientific. As yet, however, no country seems to put as much investment into protecting itself from natural disasters as from outside invaders. Thus we shall probably see an expedient solution to the Managuan problem in that the zone in which the damage was greatest will be avoided in rebuilding. A similar policy was followed at Agadir. There is no scientific justification at all for this. No one knows whether stress has been relieved in the surrounding regions. Besides which, many of the destructive earthquakes at Nicaragua are deep (that in 1926 was at a depth of 150 km). Moving the site of a city by a few kilometres can do nothing to reduce damage from earthquakes like these.

It goes without saying that the inevitability of earthquake damage in the now well recognized seismic regions makes a mockery of the attempts to organize emergency relief for damaged cities such as Managua. The surprise with which well-meaning governments repeatedly find themselves making allocations of inadequate funds for purchases of food and medical supplies is all too reminiscent of the astonishment with which the British government reacts whenever there is a heavy fall of snow in Scotland. The truth is that the world must learn somehow to live with or to survive as best it can the natural disasters which, in the nature of things, must repeatedly recur in vulnerable places. And it is now clear that only the United Nations is sufficiently well placed to organize the kind of emergency aid that will be needed for as long as cities such as Managua are vulnerable to earthquakes—and cities such as Calcutta to typhoons. Much has been heard, in recent years, of schemes like this but nothing so far has happened.

Poor Substitute for Annan

ONE of the last acts of the last Labour government in 1970 was to set up a committee to inquire into the future of broadcasting in Britain after 1976. The then Minister of Posts and Telecommunications, Mr John Stonehouse, when announcing the formation of the committee, to be headed by Lord Annan, spoke of the "breathtaking technological environment" in communications which had come to exist. The scope of the Annan Committee was therefore to be broader than of those in the past: Pilkington in 1960, Beveridge in 1949, and Ullswater in 1936. Technical changes might even, he conjectured, allow the quasi-monopoly of the broadcasting organizations to disappear.

One of the first acts of the Conservative government was to disband the Annan Committee. Not that Mr Christopher Chataway disagreed with the enthusiasm of his predecessor for the new technology (although he never risked being teased for his effusiveness). But he did not think that it was the best way to approach the decisions of 1976, when the various legal instruments authorizing the British Broadcasting Corporation, the Independent Broadcasting Authority and the various private commercial companies selling wired (or cable) television services expire. At the time Mr Chataway saw two obstacles to another Pilkington. First, British broadcasting was

falling into a pattern of ten-year cycles punctuated by full-scale inquiries; during the last years of the cycles nobody wanted to decide anything pending the outcome of the inquiry. Second, the technological possibilities were now genuinely novel. There were satellites, multi-channelled cable television and video-cassettes. The best thing, so Mr Chataway argued, was to revive the ministry's Television Advisory Committee, let it spend a year delineating the new alternatives on a purely technical basis and then the government could weigh the political and social advantages of using any of them to modify or reform present broadcasting arrangements. The TAC, under the chairmanship of Sir Robert Cockburn, was to report by January 1972, in order that there would be sufficient time for study before legislation needed to be drawn up.

Things have not turned out that way. The TAC's report has just emerged, a year late. It is provincial, conservative and misleading. Far from describing any opportunities for change, breathtaking or not, it spends most of 21 pages hashing over the BBC's and IBA's technical problems in extending UHF 625-line television coverage to as large an audience as could receive the old 405-line VHF signals now being abandoned. It takes as a basis the Pilkington Report's recommendations that six channels of national television are still the country's objective, and in fact could almost have been written by BBC engineers 12 years ago.

Here briefly are the committee's conclusions on the new communications technology. 1. A fourth television channel, using UHF and a 625-line standard, is immediately available. 2. About 1985, two extra television channels could be added by using the two broadcast bands on VHF which will be vacated when the current 405-line black-and-white transmissions of BBC-1 and ITV are ended and the transition now in progress to the UHF 625-line standard is completed. 3. Television broadcasting direct from satellites cannot be expected until the 1980s and could provide four programmes of national or near-national coverage. 4. Wired television on a national scale costs too much to be considered in the 1976 decisions. 5. Wired television can, however, help distribution of programmes in remote and rural regions and it could be used to provide local television programmes. 6. Video-cassettes, because of lack of standardization, are unlikely to come into domestic use in the period likely to be affected by the next franchise period (assumed to be twelve years).

In other words, the much touted communications technological revolution is a long way off and expensive to boot. Hence no need to change. Such conclusions should not be surprising from a committee constituted as the TAC has been, heavy with representatives of the *status quo*—the BBC, the IBA and the Post Office are all threatened by the new technology, particularly by cable television. It is no accident that nowhere in its report does the TAC mention the primary advantage of wired television, that it offers a solution to the basic problem of British broadcasting—a shortage of frequencies to accommodate the varieties of radio and television programmes that people would like to receive.

The recommendation that the VHF should be "re-engineered" in 1985 for two extra channels of television is an idea that has been kicking around the ministry for years, certainly since Pilkington. Yet nowhere does the TAC report mention the fact that the VHF is the most

desirable part of the spectrum for land mobile communications, a fast-growing service in the United States and one which is sorely lacking in the United Kingdom. Nowhere does the report mention the possibility of using the vacated VHF for more radio, nor does it mention the growing demand for the use of radio and television for specialized audiences. The Open University, for example, is not mentioned in the report. Yet the Open University has illustrated the need for extra channels at peak viewing and listening hours. The university's students want to tune in just when the BBC's biggest popular audiences also want to receive their programmes. The Open University could well expand by using radio instead of television—especially if rumours are true that those students of the Open University who are doing best are those who live in areas where BBC-2 television is unobtainable.

The TAC report shows the fallacy of believing that there is such a thing as pure technology. It protects the interests of those who wrote it, without bringing together the kind of plain factual information that politicians and the public should have before facing 1976. One would never believe, if this report were a guide to communications technology, that cable television systems can carry signals in two directions, that they are expected, in the United States, soon to distribute the mail and facsimiles of documents in large cities. Nor would one know that direct broadcasting from satellites was real enough for India to plan to use it to give educational television to 5,000 villages in a few years' time. Nor that there is an alternative to Britain owning its own satellite or helping to build a European one: Latin America is receiving a constant flow of television from Spain and Portugal because a share of Intelsat's satellites has been rented, and Brazil intends to do the same soon for a domestic educational broadcast network. Nor that in France the state broadcasting monopoly and the PTT are concerned enough about the potential of cable television to have set up a joint enterprise to study it.

What was the TAC's job if not to put such information on the state of the technology before the decision-makers now? Members of Parliament or executives of the BBC cannot be expected to pore over the fine print in *Aviation Week*. But one unforeseen benefit of this timid, self-protecting report could be to diminish the importance of 1976. The report would have it that no great technological changes need alter the *status quo*. The truth is the opposite—that the changes are tremendous and that the pressure of, say, international satellites plus multi-channelled local wired distribution will be felt all over Europe by 1980. But 1976 is too soon to make profound changes in the present broadcasting systems, and if this report has bought time it is worth something. What should be done in 1976, in fact, is to agree on a temporary continuation of the *status quo* but to permit much more experiment outside the two broadcasting authorities. The local television experiments in Greenwich, Bristol and several other cities are a good thing. Satellite broadcasts should be tried; so should two-way cable systems (that installed at Bletchley is supposed to contain two-way capacity). So should public-access television. Those are political decisions, to be sure, and if more technical advice is needed before they can be taken, it should be sought from objective consultants, not from the vested interests who stand to lose most from change.

Science Overlooked

THE British government has been commendably resolute in its efforts, in the past few months, to make Britain a part of Europe again, but its arrangements for celebrating the occasion have been curiously haphazard. Altogether, something like £300,000 has been spent on what is somewhat euphemistically called a "Fanfare for Europe"—a programme of concerts, theatrical performances and exhibitions devised by a committee with Lord Goodman as chairman. It may be that Lord Goodman is the best of all Pooh-Bahs, but he seems to have fumbled badly. Although the objective seems to have been to draw attention to the cultural relationship between Britain and the mainland which has existed for the past several centuries and which has now, with luck, become stronger, the programme which has been devised (*Fanfare for Europe*, Arrow Books, £0.70) is almost entirely innocent of attempts to demonstrate that many of the most important links between Britain and the rest of Europe have their roots in science. The nearest that the official programme comes to a recognition of the importance of science is an exhibition of ancient boats, recently dug up, at the National Maritime Museum. For the rest, there is not merely plenty of good music but a competition in karate between Britain and France, a basketball tournament, a volleyball match between teenage teams from Scotland and Belgium, an exhibition of stamps and other sporting contests, in fencing and weight-lifting, all too familiar as a means of disrupting international relations, not of cementing them. It is true that Lord Goodman's committee may not be the one solely responsible for the entire neglect of science in this programme of events—Lord Mancroft, a famous after dinner speaker, had a parallel committee responsible for what are called "other events", the fencing and the weight-lifting. The most charitable explanation is that science fell between the two committees; the most likely is that it was forgotten.

What might the committees have done? Especially with the Copernicus quincentenary, even the good Lords Goodman and Mancroft might have been moved to mark the reunification of Britain with Europe by recalling the time when the foundations of modern science were constructed by Tycho, Kepler, Copernicus, Galileo and ultimately Newton. They might also have remembered the several periods in European history when the scientific community has been more strongly welded together than any other community, with the possible exception of the Catholic Church. Did Davy and Faraday go gallivanting about France during the Napoleonic Wars for nothing? And is there not at least something favourable to be said for the way in which there has grown up several strong scientific institutions in Europe, of which CERN is the most conspicuous example (and several bad ones as well), whose work deserves some recognition in what is supposed to be a celebration of cultural links.

The neglect of science in *Fanfare for Europe* is not merely a debating point. Given the Prime Minister's devotion to music, it may be held that music, even pop music, is what the occasion called for. And it is certainly true that literature is also dealt with skimpily in the Fanfare, as are the humanities. But the overriding issue is whether it is in any sense proper that when people, even Lords Goodman and Mancroft, think of culture, they should

think first of those kinds of culture which can be most easily exhibited by means of a few telephone calls to impresarios, theatrical agents and museums. The truth is that the contribution of European scholarship in general and European science in particular to the fabric of modern life is as much cultural as practical and is on all counts indispensable. To forget this is often forgiven but is unforgivable.

In practical terms, what *Fanfare for Europe* might have included is an exhibition to show how the foundations for modern science were laid in Western Europe, how the Cartesians raised the banner of applied mathematics, how people such as Gauss and Huyghens were able to conjure up insight out of thin air and how the experimentalists such as Volta, Lavoisier, Black and Faraday (to mention only a few) created the sense which now runs through science that a theory is above all a concept to be tested. And if by any chance the organizing committee had run short of interesting topics with which to deal, there would always have been the half century which began with Darwin and Maxwell and which, by the first decade of this century, had reconstructed modern science and had drawn the whole of Europe into the enterprise. Even to imply by default that these great happenings are not cultural is to deny what everybody knows—that the way in which all people regard the world they live in has been transformed as much by scientific discovery as by any other intellectual process with its roots in the Renaissance. To be sure, it is often harder to make all this come alive than to remind people that Beethoven was a genius, but that is no excuse for not trying. The simple truth, however, is that between them Lords Goodman and Mancroft have done a shallow job. In many ways, the Prime Minister might have been better advised to have substituted for *Fanfare for Europe* a public holiday on January 1, widely kept as such in any case.

100 Years Ago



NOTES

WE believe that a reply has been received from the Government on the subject of the Arctic Expedition, which goes far to justify all that was said in our leader last week on the subject; for although the Government does not refuse absolutely to comply with the wishes of the deputation, all action will, unless strenuous efforts are made, be postponed for a year. We repeat that the deputation did not represent Science so broadly as it ought to have been represented; and we add, that if the Government thought so, it was, in our opinion, perfectly justified in refusing the demands made upon the national purse. To a certain extent, what happened in the case of the Eclipse Expedition of 1870 has been now repeated. Our readers will recollect that on that occasion the mere personal application of the Astronomer Royal was at once very properly refused, while a proper representation by the leading Societies was at once as promptly acceded to.

From *Nature*, 7, 188 and 189, January 9, 1873.

OLD WORLD

Too Much Freeze

MORALE among scientists working in government laboratories is lower than it has ever been, according to the Institution of Professional Civil Servants. Scientists are disenchanted according to Mr William McCall, general secretary of the IPCS, because of the government's constant refusal to set up an independent committee to determine the criteria by which pay for scientists within the civil service should be determined.

The government claims that pay research, by which pay of scientists within the government is compared with the pay of scientists working in industry and non-government establishments, is the most appropriate way of determining pay scales for its scientists. The IPCS, on the other hand, following many years of disagreement with the principle of pay research for scientists which culminated in the summer of 1971 with mass meetings and an independent tribunal, has vowed that there will never be another pay research exercise for government scientists.

A way out of this impasse was first put forward by the IPCS in August 1972, when it suggested the setting up of an independent committee. As yet, the government has not acceded to the request, with the result that both the IPCS and its members are becoming impatient. The IPCS has said that it will abide by the decisions of the independent committee even though it may recommend that pay research is indeed the best way.

At present, the science category is bottom of the pay league within the civil service. In 1969 the Administration, Science and what is now known as the Professional and Technology categories, had comparable maximum salaries for the Administrative Principal, Principal Scientific Officer and Principal Professional and Technology Officer grades, but since then the PSO has been left sadly behind. The Administrative Principal now commands a salary of £4,708 at the top of the scale, the Principal PTO £4,760, while the PSO has a maximum of £4,387.

The decision on whether or not to set up the committee has been affected by the government's pay freeze, and it is understood that the extended delay in making a decision is due to differences in opinion between government departments. The IPCS maintains that there is no conflict between the proposal to set up the committee and the government's

pay freeze, for the committee is to be concerned with criteria for determining pay, not with awarding increases.

The Civil Service Department said this week that it was "actively considering" the IPCS's request. Mr William McCall also said this week that the institution would have to consider the possibility of action—even stronger than that taken in 1971—if the government did not respond satisfactorily.

ROYAL SOCIETY

Research in Schools

THE Royal Society supported a hundred research projects in schools last year through its Scientific Research in Schools Committee. The scheme—which has been running since 1957—exists to enable school science teachers to undertake original research; it is financed by the Atomic Energy Authority and six companies who provide about £3,000 a year.

The committee receives about thirty applications a year from teachers and approves about twenty-five of them, providing applicants with items of equipment that their schools cannot supply. Although the grants are usually small—normally less than £100—the committee has been known to give grants of up to £600 to buy items such as a spectrophotometer. The object of the scheme is to encourage bright science graduates to enter teaching by providing them with the facilities for research if they have the time and the necessary skills. When a teacher applies for a grant he is sounded out by an adviser from his local university, who is on hand to help throughout the project.

Mr N. A. W. Le Grand, the secretary of the committee, points out that the Royal Society is anxious to support as wide a spectrum of projects as possible; current projects range from studies of pollution in the Tees Bay area to the measurement of the stability of metalamine complexes to work on radio astronomy and colour blindness. The Royal Society encourages the recipients to publish their results, and last year thirteen papers appeared in the literature as projects were completed.

Each grant is made personally to the teacher and not to his school, but the Royal Society examines the facilities of the school and the support it is likely to give the teacher before it makes a grant. The society does not automatically turn down applications from teachers who cannot involve any of their

pupils in their work, but such involvement is encouraged and one effect of the scheme has been to interest pupils in research, leading them to read science at university.

The time scale of the projects varies enormously. One on corrosion has been running for ten years, some involve seasonal studies for a number of years but other are relatively short lived. Since publicizing the scheme more widely two years ago (thousands of leaflets were mailed to schools and colleges of education all over the country), the Royal Society has been particularly eager to support practical research projects rather than pure research, which it feels is usually the province of the universities. The results have included a redesign of the scrubbing brush, which has been taken up commercially, a receiving station to pick up pictures from weather satellites, and a pram alarm that could prevent baby snatching. But the society is also supporting many more basic projects.

METEOROLOGY

Watching the Sun

from our Soviet Correspondent

AN unofficial "weather prophet" from Gornaya Shoriya in the Soviet far east has won official recognition for his theories of long range forecasting—that solar activity influences weather not only through variations in heat flux but also through other forms of radiation and that sun spot activity and variations in the solar wind should also be taken into account in long range forecasting.

Anatolii V. D'yakov, who mans the tiny meteorological station of Gornaya Shoriya assisted only by his wife, has for several years been issuing unofficial forecasts, particularly to the agricultural planners of the Virgin Lands. Although the recipients of these forecasts found them more than satisfactory ("Do we use them? We are guided by them!," commented Academician A. I. Baraev of the All-Union Institute of Agricultural Sciences), the director of the Hydrometeorological Centre, Professor B. A. Bugaev, considered them little more than "charlatanism".

The success of D'yakov's three month forecasts, however, attracted considerable press coverage, first in local Siberian journals and finally (September 27, 1972) in *Pravda*. At the end of October D'yakov was invited to address an All-Union conference on the problem of the interaction of the Sun and the lower atmosphere. This conference resolved *inter alia* to implement a research programme on the effect of

solar activity on the weather, stressing that "the rational approach to long range weather forecasting should take into account the whole totality of the perturbing factors and their variation in time". A working council to consider the problem of solar effects has been set up to work in collaboration with the Principal Geophysical Observatory (on long range forecasting) and the Hydrometeorological Centre (on short range forecasting). As part of the programme D'yakov is to prepare his own long range forecasts for 1973-74 "by way of experiment" and to submit them to the Hydrometeorological Service "for appraisal". The working council also consider it "suitable" to transfer the Gornaya Shoriya "helio-meteorological station" to the jurisdiction of the Soviet Academy of Sciences or the Hydrometeorological Service.

REACTORS

European Neutrons

BRITAIN has bought a one-third share in the Franco-German high flux reactor at Grenoble for £10 million. British participation in the reactor project comes after negotiations conducted by the Science Research Council on behalf of the government, which has provided the SRC with the necessary funds to participate in the scheme. The SRC will spend almost £2 million a year in running expenses and a further £1 million a year for ten years in capital.

The agreement, which took effect on January 1, provides that the SRC will have one-third representation on the Steering Committee, the Scientific Council and the Audit Board of the Institut Max von Laue-Paul Langevin (ILL) where the reactor is situated, and provides that the director of the ILL will be alternately German or British. British scientists will have a fair share of scientific posts at the institute, and preference will be given to British companies in the placing of some of the contracts for future work.

The SRC began negotiations with the French and Germans in August last year, following a decision by the British government not to build a high flux reactor in Britain (see *Nature*, 239, 60; 1972). The SRC Committee for Neutron Beam Research originally decided that Britain would benefit more by building its own reactor at a cost of £22 million than by joining France and Germany at Grenoble, but the government turned down the council's request for funds. The cost of the new arrangements will be only half that of a solely British reactor, but British scientists will have to be content with only one-third of the experimental time that they would otherwise have had.

The United Kingdom Atomic Energy

Authority said this week that some of its long term neutron beam experiments will be transferred to Grenoble, possibly this month, but a spokesman pointed out that the Grenoble reactor—which went critical in August last year—does not have the irradiation facilities envisaged in the proposals for the British reactor. "There is therefore a growing need to find alternative irradiation facilities."

One of the alternatives could be the construction of another high flux reactor by the three countries, but this time in Britain as provided for by a clause in the SRC's agreement.

COMPUTERS

Watching a Watchdog

THE Civil Service Department has created a Computer Agency Council to advise on the development of government data processing, and "to define areas where action is required by the Central Computer Agency . . . to promote the efficient application of processing systems in government departments".

This is the latest step in a rationalization of the previously complex machinery involved in the purchase of computers for use in government departments. Until the Central Computer Agency was set up in April last year, a few months after a recommendation by the Select Committee on Science and Technology (see *Nature*, 236, 254; 1972), the responsibility for this was shared between the Civil Service Department, the Department of Trade and Industry and the Stationery Office. But now some 600 civil servants, under the direction of Mr Stephen Spain, carry out the same functions within the Civil

Service Department itself. (Of these, about 300 work at the agency's Central Computer Bureau at Norwich, which provides a computing service for those government departments that require it, about twenty-five are responsible for policy planning, and the remainder are concerned with purchasing, contracts and so on.)

At the end of 1971, 213 computers were installed within government departments—45 per cent of them for scientific purposes—and the present rate of government expenditure on computing facilities is about £35 million a year.

The new Computer Agency Council will initially have twelve members and the chairman will be Mr Kenneth Baker, Parliamentary Secretary at the Civil Service Department. Seven of the members, including Mr Spain, represent government departments, and the other five are drawn from non-government bodies which have experience of the use of computers. Among them are Sir Brian Flowers, chairman of the Science Research Council, and Mr C. P. Williamson of British Petroleum. The Civil Service Department says that, through the council, it hopes to be able to benefit more fully from expertise in the private sector. The council is expected to have its first meeting later this month.

SOVIET SCIENCE

Interkosmos Programme

from our Soviet Correspondent

THE launch on December 1 of the latest COMECON satellite, Interkosmos-8, carrying ionosphere research experiments provided by Bulgaria, East Germany, Czechoslovakia and the Soviet Union, was the occasion for a special interview in *Pravda* (December 2, 1972) with Academician Boris Petrov of the Soviet Academy of Sciences on the whole purpose and future of Interkosmos. Academician Petrov is a leading figure in both the Soviet and the Interkosmos space programmes.

According to Academician Petrov, the chief aim of the Interkosmos programme is to gather information on the solar and galactic flux, cosmic rays and micrometeorites. The investigations have, so far, covered the active regions of the ultraviolet and X-spectra (Interkosmos 1, 4, 7), low-energy cosmic rays (Interkosmos 3 and 5), and ionosphere and magnetosphere processes.

Interkosmos-8 is continuing the ionosphere investigations of Interkosmos-2, but in contrast to its predecessor it is investigating the magnetic poles and the aurora. It is hoped that this will enable the connexion between geophysical and geomagnetic processes to be traced more clearly.

New Year Honours List

In the Honours List announced this week, D. A. K. Black, Professor of Medicine at the University of Manchester; Professor Theodore Crawford, Director of Pathological Services at St George's Hospital and Medical School; Eric Eastwood, Director of Research, General Electric Company Ltd; and F. G. Young, Professor of Biochemistry at the University of Cambridge, were made Knights Bachelor. Professor Herman Bondi, Chief Scientific Adviser to the Ministry of Defence, and Professor Claus Moser, director, Central Statistical Office, Cabinet Office, were made Knights Commander of the British Empire; and Miss Kathleen Kenyon a Dame Commander of the British Empire.

NEW WORLD

AAAS at the Crossroads

from our Washington Correspondent

ALTHOUGH eight people were arrested during a clash with police, the 139th annual meeting of the American Association for the Advancement of Science, held in Washington last week, was one of the quietest for several years. Members of a radical group of scientists known as SESPA (Scientists and Engineers for Social and Political Action), which campaigns under the slogan "Science for the People" and which has added a touch of colour, if not of harmony, to the previous three AAAS meetings, were notably thin on the ground—there were no tomatoes thrown at politicians or stabbings with knitting needles—events which have grabbed the headlines at past AAAS gatherings.

Instead, participants in the meeting were confronted with an array of lectures and symposia dealing with issues ranging from pollution control, economic growth and social responsibility to esoteric discussions of research results. Like the annual meetings of the British Association, the AAAS gatherings have become notably more political in the past few years—due in part to the proddings of SESPA—and they are increasingly running up against the knotty problem of what function they are meant to fulfil. But the AAAS, again like the BA, may itself be destined for yet more changes, for later this year a meeting will be held to discuss the future of the association and its annual meetings. That, indeed, was one theme which was often discussed last week, both in the programmed sessions and outside them.

As for the meeting itself, the event which led to the arrests was an extremely silly affair. The AAAS board of directors decided in advance of the meeting that it would take a stronger line this year against any radicals who disrupted sessions, and as a first step it forbade any organization not affiliated to the AAAS to set up literature stalls or distribute leaflets. SESPA decided, however, to ignore the ban and, as it has in the past, set up a stall in the registration area from which to peddle its wares. After a day of bickering, Dr Richard A. Scribner, the meeting director, called in the police to remove the table. Scuffling broke out and seven scientists and a reporter were arrested. SESPA screamed about freedom of speech, but Scribner replied that everybody has a chance to be heard in the meetings, and that rules are rules. In the meantime,

SESPA was offered, and later grudgingly accepted, a table in a less conspicuous place. The result: AAAS officials provided SESPA with great publicity and eight people have been charged with disorderly conduct.

Another potentially explosive situation was headed off later in the week when Dr Daniel P. Moynihan decided for the second year in succession to miss a meeting rather than face an unruly audience. Moynihan was due to chair a meeting on public policy and social science, but cried off because he said he had to go to a briefing at the State Department on his new appointment as Ambassador to India. He was, however, bitterly attacked in a leaflet distributed throughout the conference in the morning—it was alleged that his work for the White House has led to cutbacks in services to minorities—and the meeting of which he should have been chairman was overflowing. Dr Harold Orlans, a member of the Brookings Institution and one of the panellists at the meeting, later rebuked Moynihan for finding an hour to have lunch with the panel, but not even half an hour to discuss his views with the rest of the meeting. The session eventually proceeded with only minor disruption.

Apart from those few distractions, participants in the meeting were fed a diet of lectures and symposia which, although increasingly concerned with issues of science and society, are still

heavily weighted towards the presentation of scientific papers supposedly dealing with topics of general public interest, but often attended only by specialists. This year's meeting was split in the ratio of about 60 per cent scientific symposia, about 30 per cent symposia dealing with issues of science and society, and the rest of the meetings were concerned with Washington-area topics, such as community education in Anacostia.

But the meeting also marked a turning point in the association's history, for at a council meeting held on the last day, a set of by-laws was adopted which will allow the new constitution to come into effect on January 15. The AAAS is at last going to become more democratic. Approved at the annual meeting in Philadelphia a year ago, the new constitution provides for the chief officers of the AAAS to be elected by mail ballot of all the members and for the council to be reduced in size and also directly elected by the membership. Previously, the president and board of directors were elected by the council—an undemocratic procedure which has long drawn sharp criticism.

Largely the work of a committee which met under the chairmanship of Dr Leonard Rieser, a physicist from Dartmouth College, who will become president of the AAAS on January 15, the new constitution drastically reduces the size of the council, from more than

AAAS Opposes the War

IN the dying hours of the conference last week, the AAAS Council took the unprecedented step of harshly criticizing US policy in South-East Asia. The council called for immediate withdrawal of US troops from Vietnam, Laos and Cambodia, and urged that a full scale study be conducted of the long-term effects of the bombing, the use of CS and of herbicides and other methods of defoliation, both on the people and the ecology of Vietnam. The AAAS council specifically threw its support behind a bill introduced into Congress last session by Senator Gaylord Nelson and Representative Gilbert Gude, which calls on President Nixon to ask the National Academy of Sciences to carry out such a study.

Although the Vietnam war has been a chief focus of the protests of radical scientists at AAAS meetings, anti-war

groups have in the past been unable to steer such strongly worded resolutions through the council. Three years ago, however, the council did set up a commission to study the effects of herbicides in Vietnam, the results of which played a part in persuading the Department of Defense to phase out the use of chemical defoliants in the war. What the association is now requesting is that a commission be set up much like the US Atomic Bomb Casualty Commission, which made a long-term study of the effects of the atomic bombs dropped on Hiroshima and Nagasaki in the Second World War. The chief sponsor of the resolution was Dr E. M. Pfeiffer, of the University of Montana, who was also chiefly responsible for persuading the council to undertake the previous study of defoliants. The war will be on the agenda while it lasts.

500 members to about 100, and makes it more responsive to the wishes of AAAS members. In the past, the council has drawn its members from the officers of the AAAS, representatives of the association's twenty scientific sections and delegates from the 300 or so societies which are affiliated to the AAAS, a fact which effectively puts the control of the association in the hands of the affiliated societies and not necessarily in the hands of paid-up AAAS members. The by-laws adopted last week, however, put the control back where it belongs—at least in theory. The net effect of the new constitution, Dr Rieser said last week, is to "provide a vehicle whereby the association is more responsive to its members, who would be more than just subscribers to *Science*". But will the newly democratized AAAS change its role, branch out into pastures new, or even change the format of its annual meetings? Like other scientific organizations, the AAAS is now having to examine its *raison d'être*, and to find ways to be responsive to the changing climate in which science is conducted. Over a hundred years ago, when the association was first formed, its role was simple—it was a primary means of communication among scientists and between scientists and the general public, but now that scientific journals, and to some extent meetings of learned societies, provide the primary channels for communicating research results, the AAAS and in particular its annual meeting are increasingly having to take on other roles.

That much was realized more than 20 years ago, for the AAAS board of directors held a meeting in 1951 to discuss the role that the association should try to play in the decade then ahead. The recommendations were that the AAAS should devote less energy to the detailed aspects of science and pay more attention to problems concerning science, technology and their relations with society, and since then there has been a gradual increase in the general symposia served up at the annual meetings. The officers of the association have now decided, however, that it is time to take another look at the aims of the AAAS and have called a second meeting for October this year. That meeting, to be called the Second Arden House Conference, will present a report to the council at the 1974 annual meeting.

Dr Rieser expects this conference to address three chief issues. How can the AAAS incorporate the technological community into its activities—at present the association is chiefly concerned with and draws its members from the scientific community? How can the association better carry out its objective of increasing public understanding of

science? And how can it play a more effective role in making sure that science is used to increase public welfare? In particular, Dr Rieser said that the conference will be concerned with the role that the AAAS should play in the legislative process.

Those questions were given something of an airing during the annual meeting last week at a symposium entitled "Professional Societies in the Process of Change". But a question often asked outside that symposium was what is the function and the product of the annual meeting itself? It is estimated that about 8,000 scientists and others passed through the meeting, some drawn by the specialized symposia, others attracted to the sessions on more general topics, but the most common opinion was that little that was new emerged.

The chief value of the meeting may be, as Dr Rieser maintains, to provide a forum in which "A lot of people of consequence engage in debate and interaction with each other". He also pointed out that the participants are drawn from all scientific disciplines as well as from outside science itself, and that is a feature which no other major conference in the United States can claim. Moreover, Dr Rieser defended the mix between specialized scientific symposia and sessions devoted to science and society on the ground that the specialized meetings are valuable in bringing scientists up to date with advances in fields outside their own.

Whatever the Arden House Conference may recommend about the structure of the annual AAAS meeting, however, it is already due for change. It is being moved away from its traditional time between Christmas and the new year. The next meeting will be in June in Mexico City, then it will move to a permanent date in late winter. The 1974 conference, for example, has been scheduled for February in San Francisco.

Elected President

ALTHOUGH the new AAAS constitution is not yet in force, the council decided to bring in one provision a year early. The president-elect was chosen by mail ballot of all the members and simply ratified at the meeting last week. The first AAAS president to be chosen directly by the membership of the association will be Dr Roger Revelle, director of the Center for Population Studies at Harvard University, who will take up office in January 1974. About 35,000 of the 130,000 AAAS members exercised their new electoral power.

INDUSTRIAL RESEARCH

New Brooms for Good

DR LEWIS M. BRANSCOMB, now chief scientist for IBM, last week launched a sober, but nevertheless effective, attack on the federal government's policies for stimulating industrial research and development. The effectiveness of his attack stems not so much from what he said but from the fact that he was at least partly responsible for putting the policies together. He was director of the National Bureau of Standards until he left to join IBM last summer. Branscomb's argument is that the Administration has failed to build on the policy basis set out in the President's Message on Science and Technology (delivered in March last year) by refusing to spend money appropriated by Congress for programs designed to help remove barriers to industrial innovation.

The chief target of Dr Branscomb's attack, delivered during a session of the AAAS meeting, was the Experimental Technology Incentives Program (ETIP), which is designed to pinpoint the barriers to innovation and experiment with methods for removing them. Jointly administered by the National Science Foundation and the National Bureau of Standards, ETIP is one of the chief products of the planning exercise headed by Mr William Magruder and other members of the White House staff in 1971 which tried to find methods to stimulate civilian research and development. He said that the ETIP programs are neither "well defined nor understood and the effect of their establishment has been to slow down excellent programs already doing the job".

Dr Branscomb announced that the Office of Management and Budget has impounded funds for the present year for the National Bureau of Standards, so that the bureau is allowed to initiate only one new program, ETIP, and that has been allowed only 14 per cent of the funds requested from Congress. The effect has been to squeeze many of the bureau's established programs, designed to solve existing industrial problems. "It is very disappointing to see a pattern of successful work in support of economic development and public protection, welcomed by the industrial R and D community, turned off and replaced by a speculative program whose basis for usefulness is still to be established", he said. A spokesman for the Office of Management and Budget refused to confirm or deny Branscomb's allegations, but said that the amounts of money to be impounded in the present fiscal year have not yet been decided for all agencies. The Administration's budget for 1974 is released later this month.

NEWS AND VIEWS

Discoveries of New Radio Stars

THE first surveys of the radio sky revealed many radio objects but none was found in the position of a star visible to the naked eye. These first radio sources were referred to as dark stars. The earliest identifications were made with optical nebulae—supernova remnants, ionized hydrogen clouds around young stars and eruptive or peculiar galaxies. Indeed a simple calculation readily shows that the blackbody radiation from the optical disk of a star, whether it is a very bright O or B star or a red giant of large diameter, would be far below the limits of detection. Stars were then dismissed as being of no interest to radio astronomy.

Many years later two theoretical papers showed that under favourable conditions radio emission might be detectable from certain types of star. Schatzman in 1958 pointed out that because the radio emission from the Sun at the time of a flare can increase by factors of 10^6 or more above the quiet Sun value it should be possible to detect the radio emission from flare stars which erupt into even more violent flare activity than the Sun. Such radio burst emission was found by Lovell at Jodrell Bank and Slee and colleagues at Sydney.

The other prediction was made by Weyman and Chapman in 1965 who pointed out that certain red giant stars had absorption lines with blue shifted structure which indicated a flow of gas from the star. Weyman and Chapman argued that this gas would be hot and would form an extensive envelope around the red giant making it detectable at short radio wavelengths; it would be more difficult to see at longer wavelengths because this envelope would be optically thick. On page 37 of this issue of *Nature* Altenhoff and Wendker report the first detection of a red giant (Betelgeuse= α -Orionis) using the 100 m radio telescope which has recently been built at the Max Planck Institute for Radio Astronomy at Bonn. Previous attempts to detect Betelgeuse have been unsuccessful or uncertain because of the poorer sensitivity of the smaller telescopes or the low frequencies used. The Bonn results show that Betelgeuse gives a signal of 8 ± 1 milli flux units (10^{-29} W m $^{-2}$ Hz $^{-1}$) at a frequency of 10.7 GHz. This flux is consistent with emission from the corona of the star. It is also interesting that Betelgeuse is an infrared source, which implies that the outflowing gas condenses to form a circumstellar cloud of dust grains.

Another group of stars has recently been discovered by Wade and Hjellming using high sensitivity aperture synthesis techniques at the National Radio Astronomy Observatory in West Virginia. These are all eclipsing binaries in which the emitting star is of spectral type B. In all cases there is clear evidence for an outward streaming of gas from the surface of the star. The stars in this class are α -Sco (Antares—the blue component of the double star system), β -Per (Algol), β -Lyr and the star associated with the X-ray source, Cyg X-1. These four sources have a quiet state flux at 8 GHz of about 10 milli flux units, but show in addition marked irregular increases in brightness which are probably associated with the ejection of material from the star and consequent changes in the radio opacity.

The radio emission from another star, No. 349 in the

Mt Wilson Catalogue, is also described in this issue. MWC 349 is rather difficult to classify because it lies behind about 10 mag of absorption in the constellation Cygnus where it most likely belongs to the Cygnus OB2 association. It is an emission-line B star which shows gas emission from its surface and has many similarities to the eclipsing binary B stars described earlier. Infrared observations suggest that it is surrounded by a dust cloud. The first radio detection of MWC 349 was made by Braes, Habing and Schoenmaker (*Nature*, **240**, 230; 1972); the new observations reported on pages 37 and 38 by Altenhoff and Wendker at 10.7 GHz and by Baldwin, Harris and Ryle at 5.0 GHz (using the 5 km aperture synthesis telescope at Cambridge) give a much clearer picture of its nature. Its radio flux continues to rise towards higher frequencies indicating that it is radiating by free-free emission from an optically thick gas cloud with an electron temperature of $9,000 \pm 2,000$ K. The Cambridge radio astronomers were able to measure the angular diameter (2.4 arc s), and thereby estimated the mass of the emitting cloud to be 9×10^{-3} solar masses. Baldwin *et al.* point to the similarity of the radio continuum properties of MWC 349 with those of the continuum source associated with the OH masering object W3 which is thought to be the HII region around a recently formed star still embedded within the parent gas cloud.

There is clearly a reawakening of interest in stars among radio astronomers who are being encouraged by the high sensitivity of the new breed of filled and unfilled aperture radio telescopes which make feasible the detection of weak emission from the circumstellar gas. The objects detected so far are a fascinating collection—red giants, blue emission line stars with P Cygni type line profiles, X-ray sources, and compact clouds of gas, molecules and dust around recently formed stars. Radio observations are likely to provide important information about the evolution of these objects.—R. D. D.

DMN and Cervical Cancer

THE finding of what might have been dimethylnitrosamine (DMN) in pooled human cervical and vaginal discharge matter by J. S. Harington and his colleagues (see page 49 of this issue of *Nature*) raises several matters that merit discussion. First, although the study involved the examination of vaginal fluid from a hundred African women, the fact that the specimens were pooled for the purposes of chemical analysis means that the report constitutes no more than a single unconfirmed observation. In spite of the care taken in the collection of specimens the possibility of contamination of one or more of them cannot be ruled out, and even if it could, one is not to know whether the DMN allegedly found in the pooled material came from several or only one woman. Nor can it be assumed that DMN found in vaginal fluid is the result of synthesis by microbial action in the vagina. For one reason or another various objects and chemical preparations find their way into the vagina so that the possibility of

contamination of the vagina by exogenous DMN cannot be excluded.

Harington *et al.* refer to the fact that, under acid conditions, nitrosamines can be formed by the reaction of nitrites with secondary amines. This has been shown to take place, for example, in the acid conditions of the stomach (W. Fiddler *et al.*, *Nature*, **236**, 307; 1972; and W. Lijinsky *et al.*, *ibid.*, **239**, 165; 1972) and during the processing of nitrite-preserved herring into fish meal (F. Ender *et al.*, *Z. Tierphysiol., Tierernähr. Fullermittelk.*, **22**, 189; 1967). It has also been shown that nitrosamines can be formed from secondary amines and nitrates under neutral conditions in the presence of nitrate-reducing bacteria, including some strains of lactobacilli, group D streptococci, clostridia, bacteriodes and bifidobacteria (J. Sander, *Hoppe-Seyler's Z. Physiol. Chem.*, **349**, 429; 1968). The pH and temperature of the infected vagina are theoretically conducive to nitrosamine formation, but there are no very obvious sources of nitrites or secondary amines. It is possible that, as in the case of saliva (personal communication from C. L. Walters, British Food Manufacturers' Industrial Research Association), nitrites are present in low concentrations in vaginal secretions and that traces of dimethylamine are formed from lecithin present in the vaginal epithelium, but in neither case are the amounts likely to be adequate for significant nitrosamine formation. Nitrates present in urine might be reduced to nitrites by the microflora of the vagina, but one would not expect appreciable quantities of urine to find their way into the normal vaginal vault. Unless secondary amines and nitrites are introduced into the vagina for contraceptive or other purposes it is difficult to envisage why either should be present there except in trace amounts.

Harington *et al.* undertook their study as part of an investigation of the causation of cervical cancer in Southern Africans among whom the disease is common. Studies of other ethnic groups have indicated a relationship between risk of cervical cancer and sexual activity, multiplicity of sexual partners, coitus with uncircumcised males and poor standards of hygiene. There is no particular reason to think that different factors are responsible among Southern Africans. The question which the possible finding of DMN in vaginal fluid raises, therefore, is whether DMN formation is a part of the mechanism by which poor sexual hygiene increases cancer risk. There can be no doubt that exposure to DMN may increase the risk of cancer development in many animal species, but there is no evidence from animal studies that its introduction in the vagina predisposes to cancer of the cervix. This is perhaps an experiment that should now be undertaken. *A priori*, however, one would not necessarily expect intravaginally administered DMN to lead to cancer development in the cervix. It is generally agreed that its carcinogenic activity depends on prior conversion to the corresponding diazoalkane and it is not certain whether the enzymes needed for this conversion are present in the cervix.

Harington *et al.* did not measure the concentration of the alleged DMN that they found, but presumably it was low. If so, one is bound to wonder whether its presence in trace amounts would have any significance at all. A feature of life today is that the sensitivity of methods of chemical analysis in many areas of toxicology is of a different order than that of the methods available for

judging biological effects such as carcinogenic activity. The finding of a potent carcinogen in trace amounts in the vagina of a woman with cervical cancer is as uninterpretable as the finding of C-type particles in a cancer of the cervix. In neither case can a cause and effect relationship be assumed or proved.

For the many reasons given here, no conclusions can or should be drawn at this stage from the reports of Harington and his colleagues. Nevertheless, the authors were right to report their findings because they might be important and others will certainly wish to set about seeing whether they are. The first hurdle is to confirm that DMN may be present in vaginal fluid under certain circumstances; the second is to show that it may be present there in biologically significant amounts; the third is to show that DMN administered intravaginally is capable of causing cancer of the cervix in animals. A fourth and most important hurdle is to find a link between the presence of DMN in the vagina and the development of cancer of the cervix: for example, a relationship has to be demonstrated between the presence of DMN and the presence of cancer, or rather an increased risk of developing cancer, of the cervix. If all these hurdles were to be surmounted, a causal relationship would seem likely, though still not proved. On the basis of the limited information available, some of the hurdles look forbiddingly high.—From a Correspondent.

Eukaryotic DNA Replication

PROGRESS in the investigation of DNA replication in eukaryotes has been confusing and messy. The involvement of a membrane site in DNA synthesis in bacterial cells, first suggested by Jacob, Brenner and Cozin in 1963, prompted a search for a similar mechanism in eukaryotic DNA replication. Two types of investigation have been attempted, producing conflicting results.

First, in cell fractionation experiments with mammalian cells, Friedman and Mueller, Mizuno, Stoops and Sinha, Hanaoka and Yamada and Pearson and Hanawalt presented evidence that newly replicated DNA (detected by pulse-labelling with ³H-thymidine) is associated with membrane material. Its buoyant density in CsCl gradients is lower than bulk DNA and it is located in the phenol-water interphase during phenol extraction. This type of experiment, therefore, suggested that DNA replication sites are located at the nuclear membrane.

In contrast, more electron microscope autoradiographic experiments suggested that DNA replication sites were distributed throughout the nucleus. Comings and Kakefuda, investigating unsynchronized human amnion cells which had been pulse-labelled for 5 min, found label over the whole of the nucleus. When cells which were synchronized in early S-phase were pulse labelled for 5–10 min, however, they found that label was concentrated at the periphery of the nucleus at the beginning of S-phase and only later was label distributed throughout the nucleus. Comings and Kakefuda concluded that initiation of DNA replication took place on the nuclear membrane, whereas replication could occur all over the nucleus. Other authors, however (Williams and Ockey, Erlandson and de Harven and Blondel), found that DNA synthesis in early S-phase occurred all over the

nucleus, but that label became concentrated around the nuclear membrane in late S-phase. No shift of label occurred during chase periods with unlabelled thymidine and later peripheral labelling represented replication of heterochromatin close to the nuclear membrane.

All this is very confusing and the basic questions remain unanswered. Does DNA replication occur solely at the nuclear membrane or over the entire nucleus? Does DNA remain fixed in the nucleus while the replication site moves along it, or does the replication site remain fixed while the DNA is displaced relative to it, or do both replication site and DNA move?

On page 32 of this issue of *Nature*, Huberman, Tsai and Deich at last throw some light on this picture of confusion, especially with regard to the autoradiography experiments. The two principal sources of error in previous work have been over-long pulses of labelled thymidine and variation in methods of cell synchronization. Because DNA replicates at a rate of 0.5–1.2 $\mu\text{m}/\text{min}$, a pulse of 2 min and more could result in erroneous location of originally peripheral grains in a central position in the nucleus. All of the previous autoradiographic studies involved pulse times of 5 min or greater.

The amethopterin method of synchronization used by Comings and Kakefuda is now known to cause cellular damage in the form of abnormal nuclear transformation. Thus Tokuyasu, identifying a cell's position in S-phase according to its centriolar development, found that the cells with heavy peripheral labelling identified by Comings as early S-phase were, in fact, late S-phase.

Huberman *et al.* carefully avoided these pitfalls. They used short pulse times of 0.5 min and the much gentler synchronization method of metaphase selection, and found general labelling throughout the nucleus in early S-phase with peripheral labelling becoming predominant in late S-phase. Because it is known that heterochromatin is late replicating, and because Huberman's pictures show a preponderance of condensed chromatin at the nuclear envelope, these results suggest that euchromatin is replicated early in S-phase and is distributed throughout the nucleus, whereas heterochromatin replicates late in S-phase and is located close to the inner nuclear membrane.

Moreover, in unsynchronized cells,

Huberman *et al.* found no difference in the location of grains after a 0.5 min pulse, a 10 min pulse and a 0.5 min pulse followed by a 6 h chase and, in synchronized cells, label from a 0.5 min pulse remained unchanged in position after a 6 h chase. They conclude, therefore, that DNA has a stable location in the nucleus.

Finally, Huberman *et al.* suggest that, in the cell fractionation experiments, it is possible that newly replicated DNA may possess special features such as extensive single strandedness which may cause it to bind more protein or membrane material than bulk DNA. Indeed, careful experiments recently reported by Fakan *et al.* confirm that the behaviour of newly replicated DNA in CsCl gradient and phenol extracts is a result of its single strandedness rather than its association with membrane material. They present evidence that newly replicated DNA is not preferentially located with nuclear membranes but is found in a fraction of chromosomal deoxyribonucleoprotein very slightly lighter than bulk deoxyribonucleoprotein and probably consisting of DNA and the additional proteins involved in DNA replication.—From a Correspondent.

TUMOUR VIROLOGY

Mutant Viruses

from our Cell Biology Correspondent

THE first report of the isolation of temperature sensitive mutants of polyoma virus appeared in 1965 and, for reasons that are far from clear, attempts to isolate similar mutants of Simian Virus 40 failed for several years. In the past two years, however, three or four groups have succeeded in isolating temperature sensitive mutants of SV40 and have begun the job of characterizing them. Tegtmeyer, for example (*J. Virol.*, **10**, 591; 1972), has characterized three of his mutants, *tsA7*, *tsA28* and *tsA30*, which are all in one complementation group and are apparently SV40 equivalents of Fried's *tsa* mutant of polyoma virus, one of the most instructive mutants of this virus. The gene that is defined by Tegtmeyer's three mutants specifies a function that is required for the initiation but not the maintenance of transformation and also for the initiation of rounds of viral DNA replication during the lytic cycle of this virus.

When non-permissive mouse 3T3 cells at 39° C are infected with any one of the three SV40 mutants, the number of cells that are transformed is some 25-fold to 158-fold less than the number transformed after infections at 33° C; the function of the mutated gene is

Direct Sequencing of Phage DNA

IN next week's *Nature New Biology* (January 10) there is a set of three reports from the MRC Laboratory of Molecular Biology in Cambridge which describe how the base sequences of two fragments of the single-stranded DNA of bacteriophage ϕX174 have been analysed directly and how one of these sequences has been identified as the 5'-amino-terminal end of the spike protein cistron of the phage genome. Ziff, Sedat and Galibert, working in Sanger's laboratory, used endonuclease IV induced by phage T4 to digest partially ϕX174 DNA and from the digestion products they isolated a fragment forty-eight nucleotides long. After this fragment was depurinated and digested with snake venom phosphodiesterase, spleen phosphodiesterase and endonuclease IV, the sequences of the oligonucleotides which comprise this fragment were determined by fingerprint analyses. These methods should prove to be of general application for sequencing DNAs.

Robertson, Barrell, Weith and Donelson have also sequenced a fragment of ϕX174 DNA using some of the methods developed by Ziff *et al.* They isolated a fragment of this viral DNA by first

binding ribosomes to the DNA and then digesting away all but the sequence protected by the ribosomes. This technique has, of course, been used to isolate ribosome binding sites of the RNAs of phages R17 and Q β preparatory to sequencing.

The sequence of the ϕX174 DNA protected by ribosome binding proved, as expected, to contain a triplet ATG, equivalent to an AUG codon; moreover, if the sequence is arranged to maximize base pairing the ATG triplet occurs at the loop of a hairpin structure. As Robertson *et al.* point out, this and other features of the sequence are shared by the sequences of the initiation sites of the R17 and Q β phage coat protein cistrons.

All these data indicate that the fragment of ϕX174 DNA that has been analysed is the beginning of a cistron. In the third article of the set, Air and Bridgen report the amino-terminal amino-acid sequence of the ϕX174 spike protein specified by cistron G. This amino-acid sequence is precisely that predicted from the nucleotide sequence reported by Robertson *et al.*, who have clearly isolated and analysed the first part of the ϕX174 G cistron.

therefore required to initiate transformation. When permissive African green monkey cells of the AH line are infected at 33° C, 39° C and 41° C with these mutants the yield of infectious SV40 particles is 10-fold less at 39° C than at 33° C and about 10⁴ less at 41° C than at 33° C. Moreover, the amount of infectious DNA that can be extracted from cells at the higher temperatures is commensurately lower. The mutant virus particles are not themselves more thermolabile than wild type virus particles, and this indicates that the mutated gene probably does not specify a structural protein of the virion, and that infections with naked mutant DNA, like infections with mutant particles, are temperature sensitive. It would seem, therefore, that adsorption and uncoating of these mutant particles are not temperature sensitive events.

To investigate further the temperature sensitive function Tegtmeier infected AH cells at the permissive temperature. After replication of the viral DNA had started he shifted the cells to 41° C and fed them 10-minute pulses of ³H-thymidine. Viral DNA was then extracted from the cells and analysed by gel electrophoresis, using a technique that Tegtmeier and Macasiet (*J. Virol.*, **10**, 599; 1972) have devised. Tegtmeier concludes, from the pattern of incorporation of label into progeny SV40 DNA during such shift-up experiments and during shift-down experiments, that at the non-permissive temperature DNA molecules partially replicated at 33° C can be completed but that new rounds of replication cannot be initiated. In other words, Tegtmeier's experiments clearly indicate that the *tsA* gene of SV40 specifies a specific initiator of viral DNA replication and presumably the same is true of the *tsa* mutant of polyoma virus. Needless to say, the protein specified by this gene has not yet been isolated, but it will not be surprising if it proves to be either an endonuclease or a protein capable of denaturing double-stranded DNA because either or both of these activities may be required to initiate viral DNA replication and integration of viral DNA into host cell DNA.

In the same issue of the *Journal of Virology* (page 653) Scolnick, Stephenson and Aaronson report the isolation of mutants of murine sarcoma virus which carry a lesion(s) in a gene(s) required for the maintenance of transformation. They obtained mutagenized stocks of Kirsten mouse sarcoma virus by exposing to bromodeoxyuridine rat cells, which were supporting the replication of this virus together with murine leukaemia virus. They then infected NRK rat cells with limiting dilutions of the mutagenized KiMSV (MLV) and isolated clones of transformants using microwell plates. By

replica plating these transformants at 32° C and 39–40° C Scolnick *et al.* detected three lines (out of 3,000 tested) of transformants which, as judged by colony morphology, were temperature sensitive for transformation. The transforming sarcoma virus genome was rescued from these non-producer cells by superinfecting them with murine leukaemia virus. Two of the three rescued sarcoma viruses proved to give rise to temperature sensitive transformants.

Hybridization tests proved that KiMSV RNA is synthesized in similar amounts in cells transformed by these viruses and grown either at 32° C or 39° C. Transcription is not, therefore, temperature sensitive. Apart from that, these mutants have not been further characterized, but Scolnick *et al.* say they intend to isolate a suite of such mutants and by complementation tests determine how many MSV genes are involved in maintaining transformation.

SPIN LABELS

Radical Solutions

from our Molecular Biology Correspondent
THE spin-label technique has been applied with discrimination and elegance to several systems by McConnell and others. For placing this instrument into so many hot and eager little hands, however, its inventors will have to answer to their consciences, for a rash of electron spin resonance (ESR) spectra is now spreading like a contagion through the biochemical literature. Spin labels being commercially avail-

able, sensitive to the smallest kind of environmental change, and the ESR experiments technically undemanding, it is now possible to generate mountains of data with rather little effort, and the comfortable conviction that even if it is by no means obvious what they mean, they must assuredly mean something.

Through the gathering fog one may, however, discern some new and potentially useful ramifications of the method, as regards, for example, the measurements of critical distances. Two unpaired spins sufficiently close together will interact, and the perturbation of the ESR spectrum can be analysed in terms of the distance between them; second, NMR signals are also apt to be broadened if the nuclei from which they arise are in close proximity to a paramagnetic centre. The second method has been explored by a group in Oxford, and an interesting approach to the potentialities of the first has now been made by Russian workers.

The story is to be found in two articles just out. Kokorin *et al.* (*Biophysics*, **17**, 34; 1972) have examined the effect of intermolecular distance on the ESR spectra of a series of iminoxyl derivatives in solution at low temperature. These conditions, including the use of glassy solvents, such as water-glycerol mixtures, are chosen to allow the observation of dipole-dipole interactions, in the absence of the otherwise important exchange contributions. As the concentration of the radical increases, the average intermolecular distance becomes small enough for dipole-dipole broadening and splitting to

Aminoacyl-tRNA Synthetase in RNA Tumour Viruses

RNA tumour virus particles have been shown to contain a variety of enzymatic activities and several sorts of nucleic acids, including some forms of host tRNAs, that are accumulated in a non-random way presumably as the virus particles bud from the surfaces of productively infected cells. These viruses also contain some ribosomes of the host cell.

It is not yet understood why the RNA tumour viruses should sequester parts of the protein synthesizing machinery of the host cell, nor is it known whether they function to make protein after they are incorporated into the virions, but the list of components discovered in these viruses continues to lengthen. Trávníček and Říman, for example, as they report next Wednesday in *Nature New Biology* (January 10), have now detected aminoacyl-tRNA synthetase in avian myeloblastosis virions.

In avian myeloblastosis virus particles lysine tRNA is more abundant than any other species of tRNA,

although that is not the case in the cells in which this virus replicates. Trávníček and Říman therefore assayed virions disrupted by the non-ionic detergent 'Nonidet' for an aminoacyl-tRNA synthetase capable of charging lysyl-tRNA with lysine, and sure enough they found such activity. Furthermore, comparisons of the aminoacyl-tRNA synthetase activities for leucine, lysine and valine in AMV particles and in host cells indicate that both lysine tRNA and lysyl-tRNA synthetase are preferentially accumulated in the virus particles.

As Trávníček and Říman comment, the selective rather than random sequestration of particular components of the host cell's protein synthesizing machinery by these viruses suggests that the process may not be fortuitous. And if it is not fortuitous these molecules must presumably function at some stage in the life history of the virus particles; precisely when has yet to be determined.

manifest itself. A simple quantitative measure of the resulting perturbation of the spectrum is the ratio of the amplitude of the central line to that of one of the hyperfine components. The broadening, and also, where it can be observed, the dipole-dipole splitting, depend on the inverse-cube of the separation, which can therefore be determined.

For a series of iminoxyl biradicals, in which the two paramagnetic centres are separated by structures of varying length, the separations so derived agree to about 1 Å with those based on the concentration calibration for monoradicals. For oxyhaemoglobin bearing a spin label on a β -chain thiol group, the line-width parameter is the same as for the free label, whereas the same radical attached to methaemoglobin displays broadening, in consequence apparently of interaction with the high-spin ferric haem iron atom. With two iodoacetate-based spin labels, one on each β -chain of oxyhaemoglobin, there is again no broadening, in accordance with the large separation of the two thiols in the molecule. If, on the other hand, a second label is introduced by way of a cyanuric fluoride derivative, which is thought to react with a histidine, interaction between this and the paramagnetic label on the thiol is observed, to an extent that indicates a separation of 25 Å. The size of the label makes this a somewhat vague parameter, and it is in any case at the upper distance limit of sensitivity. When the protein is denatured, however, the separation appears to decrease by 3 Å.

In an accompanying article (Kulikov *et al.*, *ibid.*, 40), the intramolecular interactions of a series of biradicals, mostly on rigid skeletons, are observed by the broadening of ESR signals from glassy low-temperature solutions, using the second moment of the spectrum as a criterion. The interaction falls off in the expected manner with separation, being very large at 12 Å, and barely measurable at 18 Å. The agreement between the separations, as calculated from the broadening and measured on molecular models, is remarkably good. Compounds containing more than two radicals, and even a copolymer of styrene, acrylic acid and an iminoxyl derivative, have also been examined in the same way. Interactions between spin labels on various groups of proteins are described; for example, the distance between the two reactive thiols needed for ATPase activity of myosin has been estimated at 14–18 Å.

A similar approach to the measurement of distances between labelled molecules in a phospholipid bilayer has been explored by Marsh and Smith (*Biochem. Biophys. Res. Commun.*, **49**, 916; 1972). Spin-labelled cholestane

introduced into bilayers at sufficient concentration shows large dipole-dipole splitting when the magnetic field is perpendicular to the planes of the stacked bilayers. The separation of the labelled molecules in the plane is calculated from the splitting, and is found (not surprisingly) to vary with the composition of the phospholipid. In saturated phospholipid bilayers the spin-labelled species are close together, whereas the introduction of cholesterol drives them apart.

This general approach to the measurement of distances between groups capable of accepting a spin label may turn out to have considerable advantages over measurement of electronic excitation transfer. Whether it too will remain a technique of primarily decorative rather than utilitarian value in regard to real biological problems will remain to be seen. One has learned to read the signs in the lofty phrases, usually to be found at the ends of papers, regarding the promise and scope of whatever new method has just been trundled onto the launching pad.

DESERT ANIMALS

Avoiding a Salty Diet

from our Animal Behaviour Correspondent

THE harsh environment of the desert poses many problems, such as those of combating heat and obtaining enough food and water, to animals like the kangaroo rats (*Heteromyidae*) which live in the deserts of western North America. The possibility of obtaining food and water from such plants as can survive there is confounded by the fact that one of the adaptations shown by desert plants such as the saltbush (*Atriplex*) is to have a layer of salt-filled

tissue at the surface of their leaves. This seems to help the salt-water balance of the plants but would make them dangerous eating for most animals because of the high concentration of salt. In one species of kangaroo rat, *Dipodomys microps*, this particular difficulty has been partially solved by the evolution of specialized teeth and behaviour which help to provide it with a year round supply of food.

G. J. Kenagy reports (*Science*, **178**, 1094; 1972) that this species has broad, chisel-shaped lower incisors, unlike other species of kangaroo rat living in the same area, which have relatively narrow and rounded lower incisors. Chisel-shaped lower teeth enable it to strip off the salty outer layers of the leaves and obtain the inner portions of the leaves which are relatively salt-free.

D. microps holds a saltbush leaf in its forefeet and draws it over its lower teeth about ten times, so shaving off the outer layers of one side. It then turns the leaf over and does the same thing to the other side. Kenagy found that the discarded shavings are very high in electrolytes and are not eaten by the rats, whereas the sodium concentration of the inner tissue, which the rats eat, was only 3 per cent of that of the shavings. Furthermore, in a feeding experiment, Kenagy found that *D. microps* could survive for at least 16 days on a diet of nothing but saltbush leaves. A closely related species, *D. merriami*, would not eat these leaves and died within 2 days.

Saltbush leaves are available all year round, so that *D. microps* is provided with a predictable and continuous food supply. This minimizes competition with closely related species which subsist on seeds, but for whom the saltbush is like the water, water everywhere of the Ancient Mariner.

Homology of Fungal and Mammalian Protein

THE sequence of the enzyme glutamate dehydrogenase from bovine liver is known from the work of Smith and co-workers. These workers also showed that there is little divergence between this and the corresponding protein from chicken, only twenty-six substitutions being found in the 500 residues. Now Wootton, Chambers, Taylor and Fincham report in next Wednesday's *Nature New Biology* (January 10) their comparison of the NADP-dependent glutamate dehydrogenase of *Neurospora crassa* with the mammalian species.

Wootton *et al.* sequenced tryptic peptides and tried to index each of them on the sequence of the bovine liver enzyme. In general a unique best-match was found, with a vanishingly low statistical probability that it would

have occurred by chance. There is a lysine in a position corresponding to the reactive lys-126 of the bovine sequence, and a general sequence homology around it extends the observation of a similar centre in the sequence of bovine glyceraldehyde 3-phosphate dehydrogenase.

Some *Neurospora* mutants were also examined, and it seems that a cluster of basic residues, his-lys-his, is somehow involved in stabilizing the enzymatically active conformation of the protein, for replacements in this position lead to anomalous activation behaviour. The existence of distinct homologies in many parts of the *Neurospora* and bovine glutamate dehydrogenase sequences is taken to signify a common evolutionary origin, rather than evolutionary convergence.

ULTRASOUND

Clinical Applications

from a Correspondent

THE multidisciplinary nature of ultrasonic techniques in medicine was well illustrated at a conference held at the Welsh National School of Medicine, Cardiff, on December 15. The value of diagnostic ultrasound in obstetrics has now been established, and the precision possible with current methods in detecting and recording the foetal heart from the seventh week of pregnancy was described by Dr H. P. Robinson (University of Glasgow). Considerable accuracy has been claimed for the measurement of the biparietal diameter of the foetal skull *in utero*, which provides important information on foetal gestation and growth. It was, however, pointed out by Dr D. Watmough (University of Aberdeen) that there are certain pitfalls for the unwary. He demonstrated that it is possible to obtain a variety of midlines corresponding to differing diameters of the foetal head. He emphasized the dangers of relying on a simple reading and concluded that both maturity and growth should be assessed by serial measurements over an extended period.

The significance of antenatal bleeding in various stages of pregnancy can be difficult to assess clinically. Dr Margaret Jones (Welsh National School of Medicine, Cardiff) showed that ultrasonic techniques have a valuable part to play in the management of patients who are bleeding in the first trimester to confirm the presence of a continuing normal pregnancy, or to detect a missed abortion or a hydatidiform mole. In the second and third trimesters accurate localization of the placental site is possible. It was pointed out that it is not sufficient to localize the inferior pole of the placenta accurately in early pregnancy as the placenta rises in late pregnancy.

Dr D. A. Carpenter (Commonwealth Acoustic Laboratories, Sydney) provided an exciting glimpse into the future of the art by describing a series of developments aimed at improving the information content without the use of computers. The resolution has been improved by the technique of film echography using a non-storage display tube. A substantial improvement was obtained in the grey scale and the amount of low level echoes displayed on the echogram. Further improvement in the resolution was achieved by the use of medium focused transducers. Further developments are envisaged, which will involve increasing scanning speed and electronic steering and focusing of the ultrasonic beam.

There is, of course, considerable interest in the use of computers in ultra-

sonic examinations, and a report by Drs R. A. Mountford and M. Halliwell (University of Bristol) of ultrasonic liver and kidney scanning with the aid of off-line computation was of particular value. They have constructed an automated system of data acquisition with direct computer-compatible output and have been able to confirm that the mean echo amplitude was considerably increased in liver cirrhosis. By using off-line computational techniques in an array scanning system, information from the focus can be digitized and stored. Subsequent analysis yields a contour type C-scan display.

Dr R. B. Pridie (London) described how the ultrasound cardiogram could measure the rate of closure of the mitral valve during diastole. The rate of closure is slow in mitral stenosis and in hypertrophic cardiomyopathy. The diastole closure rate is increased in mitral incompetence and in congestive cardiomyopathy. He concluded that if

the pulmonary vein pressure is known, a measure of the left ventricular compliance could be obtained using the diastolic closure rate of the mitral valve as the only other parameter.

The manner in which a tissue returns ultrasonic echoes may reflect features of its structure. Drs R. C. Chivers and C. R. Hill (Institute of Cancer Research, Sutton) described an experimental study involving a new approach in which the spectral distribution of the returning echoes from various tissues was analysed.

Japanese workers have for some time described the use of ultrasonic techniques in the detection of gall stones. These results have been confirmed by Dr I. H. Gravelle (Welsh National School of Medicine, Cardiff). He described how gall stones could be diagnosed, but pointed out that small stones could not at present be detected unless grouped together. The method can be used to treat

Origins of Malignant Lymphoid Cells

THE discovery of distinctive cell surface antigens on thymus-dependent lymphocytes (T lymphocytes) and thymus-independent lymphocytes (B lymphocytes) has provided methods for investigating the organization and function of normal lymphoid tissues. Distinctive cell surface antigens on T and B lymphocytes are detected with specific allo or hetero-antisera and have been identified in several species including chicken, mouse, rat, guinea-pig, and man. Recently such antisera have been used to identify the possible origins of malignant lymphoid cells.

One such study of lymphomas induced in chickens by Marek's disease herpes virus is reported by Hudson and Payne in *Nature New Biology* next week (January 10) and indicates that most lymphoid cells within the lymphomas carry T lymphocyte antigens. This, and their previous observation that chickens having only T lymphocytes (achieved by neonatal bursectomy and X-irradiation) failed to develop lymphomas after infection with Marek's disease virus, suggests that the target cell for the virus is a T lymphocyte. Further support for this hypothesis must await an answer to the question whether or not the T cells in the lymphomas are transformed and carry virus-induced tumour-specific antigens. The RNA leukaemia viruses also induce lymphomas in chickens, but the target cell in these lymphomas is probably a B lymphocyte, for early bursectomy prevents lymphoma induction (Cooper *et al.*, *J. Nat. Cancer Inst.*, **41**, 373; 1968).

Murine leukaemias have been examined for T lymphocyte alloantigens

(θ , TL, LyA, LyB, LyC, LyD) and for B lymphocyte hetero-antigens (MBLA) and surface immunoglobulins (Ig). Many of the murine leukaemias induced by the oncogenic RNA viruses have θ , TL, LyA and so on, and are thus likely to be of T origin. But very few murine leukaemias have been identified which lack T markers yet have B markers. The murine myelomas do not have T markers but do have B markers such as the alloantigen PC, the heteroantigens MSPCA and MBLA, and surface Ig (Shevach *et al.*, *J. Immunol.*, **108**, 1146; 1972).

Markers for human T and B lymphocytes are only now becoming available. The best characterized marker is surface Ig, which is detected on B lymphocytes with anti-Ig serum (20 to 30% of normal blood lymphocytes have surface Ig). Most of the blood lymphocytes of patients with chronic lymphatic leukaemia (CLL) have surface Ig of the IgM class and lack T antigens (as detected with hetero-antisera) (Wilson and Nossal, *Lancet*, ii, 788; 1971). It seems likely that CLL is a disease of the B lymphocyte series. Because T lymphocyte markers are not yet readily available or standardized, the origins of lymphoid malignancies lacking surface Ig have not been determined.

The data so far available suggest that lymphoid malignancies may arise at several stages during lymphocyte differentiation. Antigenic characterization of malignant lymphoid cells will provide an additional means of classifying these diseases, and may provide a basis for studies of interactions between carcinogen and target cell.

patients with obstructive jaundice.

Finally, the conference had a timely reminder from Dr Mary Dyson (London) of the possible risks of ultrasonic scanning. She described experiments in which chick embryos were irradiated at the head process stage. Certain dose parameters produced a significant increase in abnormalities, particularly of the central nervous system. At diagnostic levels no harmful results have been reported, but the field is not yet fully explored.

CHEMISTRY

Asymmetry of C-F

from a Correspondent

RECENT work on the fluorine n.m.r. spectra of silver trifluoroacetate, $\text{Ag}^+ \text{CF}_3\text{CO}_2^-$, by Waugh and his colleagues at MIT (*J. Chem. Phys.*, **57**, 2147; 1972) has revealed a remarkable lack of axial symmetry in the chemical shift tensor for the C-F bonds.

A typical C-F bond has axial symmetry, that is all directions perpendicular to the bond are equivalent. More specifically, vector properties such as a bond dipole moment contribution will lie parallel to the bond; likewise a second rank tensor associated with a property such as the optical polarizability is expected to have one of its principal directions parallel to the bond and the other two to be associated with equal principal values and consequently referring to all directions perpendicular to the bond. Such axial symmetry is a necessary property of a model of a bond in isolation, and would be expected to be realistic for real bonds in molecules, at least in the absence of π electron or aromatic effects.

To understand the findings it must be remembered that an n.m.r. chemical shift, the quantity σ in the energy expression, $-\hbar\gamma\mathbf{I}(1-\sigma)\cdot\mathbf{B}$, relates to the departure of the n.m.r. frequency for the substance being studied from that of the bare nucleus or other reference state. For fluorine, σ may reach several hundred p.p.m. and is well known for many liquids. But for liquids the quantity observed is the mean value of σ , that is one-third of the trace (Spur or diagonal sum) of the tensor quantity. With very few exceptions these mean values are the only quantities that have been available. The fuller information could not be obtained from solid state spectra because it is normally obscured by the dipole-dipole coupling between nuclei; this is a much bigger effect than the chemical shift and leads to line widths of the order of 10 kHz.

Waugh and his colleagues used a special sequence of radiofrequency pulses to excite the resonance and to remove the effect of the dipole-dipole

coupling. The line widths were 0.5 kHz or less, thus enabling the details of the chemical shift to be made plain. In a single crystal of $\text{Ag}^+ \text{CF}_3\text{CO}_2^-$, at least at 40 K where the $-\text{CF}_3$ rotational motion has been frozen, each distinct C-F bond in the unit cell may make a different angle with the magnetic flux density and consequently have a different effective chemical shift for the fluorine resonance. Six lines were resolved and measurements at several crystal orientations with respect to the magnetic field allowed the complete chemical shift tensor to be derived for each of the C-F bonds of the $-\text{CF}_3$ group. There are small, yet real, differences between the bonds, but essentially the results indicate, for each C-F bond, that the principal values and principal axis directions of the σ tensor are +73 p.p.m. along the bond, -75 p.p.m. in the CCF plane and +2 p.p.m. in the circumferential direction around the $-\text{CF}_3$ group.

Such a tensor is clearly not of the expected axial type, which would require principal values in the ratios +74: -37: -37. This is surprising, for the electrons in the immediate vicinity of the nucleus normally dominate its chemical shift. Theoretical chemists will have to get to work to explain the lack of axial symmetry and the differences between the three bonds, and to find the reason why the principal direction is actually at an angle of 10° to the C-F bond direction rather than strictly parallel to it.

Oncornaviruses and Breast Cancer

Two of the chief obstacles in the way of further investigations of the virus-like particles which have been detected in samples of human milks and may be human counterparts of mouse mammary tumour viruses are the lack of cultivated cells which can be productively infected or transformed by these milk particles, and the lack of cultures of human breast cells that produce the particles. At present these cells can be obtained only from human milk and there is no assay for their biological activity. The experiments reported in next Wednesday's *Nature New Biology* (January 10) by Keydar *et al.*, however, may show the way to a method of obtaining reproducible cultures of human cells that support the replication of the human "milk virus".

Keydar and his colleagues co-cultivated primary human embryo cells and human breast cancer cells, and exposed other cultures of human embryonic cells to milk, from breast cancer patients, which had reverse transcriptase activity and presumably contained the milk particles. In nine out of a

NUCLEAR PHYSICS

Exchange Effects

EVIDENCE for the occurrence of substantial exchange effects in the elastic scattering of oxygen-16 nuclei from oxygen-18 nuclei is given in a recent issue of *Physical Review Letters* (**29**, 1683; 1972) by Gelbke and his colleagues of the Max-Planck-Institut für Kernphysik at Heidelberg.

It has long been known that, when a light nucleus (typically of mass less than 40 a.m.u.) is scattered from a target composed of similar light nuclei, the observed distribution in angle of the bombarding particles cannot be explained solely by an interaction in which the incident particle is affected by a combination of the Coulomb repulsion between the particles and a nuclear interaction simulated by a potential well with a real and imaginary part (an optical model potential). What the extra mechanism is or what quirk of the mechanism makes the observed angular distribution of elastically scattered heavy particle deviate from that predicted by the optical model has been the subject of considerable speculation. The work of Gelbke *et al.*, however, eliminates some of the suggested possibilities and comes out in favour of an exchange contribution.

The telltale feature of anomalies of this kind in elastic scattering is their variation with the energy of the incident particle. The Heidelberg team studied the reaction of ^{16}O on ^{18}O at 24 MeV.

total of fifteen such cultures Keydar *et al.* detected the production of particles which contain 60-70S RNA and reverse transcriptase activity and have a buoyant density of 1.18 g/ml. These are, of course, diagnostic properties of RNA tumour viruses. Furthermore, some of these cultures have been producing these particles for nine months whereas control cultures of human embryonic cells not exposed to milk or co-cultivated with cancerous breast tissue have not produced any detectable particles with the characteristics of RNA tumour viruses.

As Keydar *et al.* point out, the particles which are being produced in their cultures may have arisen from the cancer cells and may be involved in the aetiology of breast cancer. On the other hand, they may be contaminants or they may have arisen in the embryonic cells. These second alternatives, however, are less likely and attempts are under way to characterize the particles produced in the cultures and establish their relationship, if any, with virus-like particles in human milks.

28 MeV and 32 MeV—energies close to and slightly above the Coulomb barrier for the interaction of these oxygen nuclei. Gelbke and his colleagues found that at these energies the angular distribution of the scattered particles has two distinct minima in intensity between 90° and 150° ; these occurred at approximately the same angle regardless of the energy of the incident particle. This observation immediately rules out explanations of the anomalous behaviour based on the absorption of the incident particle by the nucleus to different extents depending on its angular momentum relative to the scattering nucleus. Thus the anomalous behaviour cannot be explained by an optical model that contains a term which explicitly depends on the angular momentum of the particles, although observations at one energy can be adequately described by such a model.

Gelbke *et al.* think that the incident ^{16}O nucleus picks up the two least bound neutrons in an ^{18}O nucleus and imparts sufficient momentum to the ^{16}O nucleus thus formed for it to emerge from the target. This ^{16}O is, of course, indistinguishable from a normally scattered ^{16}O nucleus.

The experimental angular distributions are fitted by a model which includes contributions from such an exchange mechanism and, as well as reproducing each individual measured distribution at the three energies, the variation with energy is also satisfactorily reproduced. Minor differences in the theoretical fits to the experimental points at the three energies are attributed to the fact that the probability of the incident ^{16}O nucleus picking up the two neutrons from ^{18}O varies with energy.

RADIATION DAMAGE

Precipitate Instability

from our Materials Science Correspondent
It is not at all surprising that an unprecedented research effort is being devoted to a form of radiation damage that is specific to fast reactors for, even though nuclear power, under pressure from environmentalists, is unfashionable, those persons responsible for national energy policy know perfectly well that in the long run most power must come from nuclear reactors, and the fast reactor in particular.

Radiation damage in fast reactors is a consequence of the fast neutrons and the high fuel temperatures: fuel swelling is caused by the formation of voids, which are themselves formed by the selective agglomeration of excess lattice vacancies resulting from neutron damage. When a neutron starts a collision sequence which causes a number of atoms to be knocked out

of the crystal lattice, equal numbers of vacancies and interstitials result. If, as is all too apt to happen, the interstitial atoms migrate faster than the vacancies, then the former quickly disappear at "sinks" and the vacancies are left high and dry; they are then likely to combine to form microscopic cavities or voids, and the fuel or the material of the fuel envelope swells dangerously. This has been a serious problem, but because of extensive research since 1967 it is now well on the way to solution.

One of the principal remedies is to use alloys containing a fine dispersion of precipitates; migrating interstitials are in some way arrested when they impinge on a precipitate particle, and are held captive there until they are neutralized by a wandering vacancy. So, in the end, almost all the interstitials recombine with vacancies and few vacancies are left to form voids. Thus, some nickel-base alloys of the Nimonic type have been found particularly suitable for the manufacture of fuel cans, for the fine precipitates of γ' (ordered Ni_3Al) impede swelling in the manner described. For this purpose, it is necessary for the precipitates to be particularly finely distributed (to increase the probability of interstitial capture), and to stay that way in service. It is this last requirement which is difficult, because all precipitate populations are subject to the law of Ostwald ripening—that is, operation of the Matthew Principle, or the growth of the large at the expense of the small.

A group of investigators at Harwell have now systematically examined, both theoretically and experimentally, the behaviour of precipitate populations in a radiation field. The Matthew Principle is counteracted by the steady destruction of precipitates by neutron damage, and the diffusion of solute atoms into the matrix at a rate which is itself accelerated by the presence of excess vacancies. From their theoretical calculations, Nelson, Hudson and Maze (J. Nucl. Mater., **44**, 318; 1972) find that bigger particles redissolve faster than small ones, and that this is true whichever of two alternative mechanisms of resolution operates.

The final conclusion is that in the presence of steady neutron irradiation, large particles dissolve preferentially but small particles on balance still grow, so that a dynamic equilibrium is established and most particles finish up close to a preferred size, which is quite small. Irradiation thus effectively neutralizes Ostwald ripening. This conclusion is confirmed by experimental observations on a Nimonic-type alloy containing coherent γ' particles, but not by observations on another kind of nickel alloy containing incoherent precipitates. The last of these are subject to normal

coarsening, because (it seems) one of the mechanisms for irradiation induced resolution of precipitates, which requires mass transport across the particle/matrix interface, does not work satisfactorily across incoherent (high energy) interfaces.

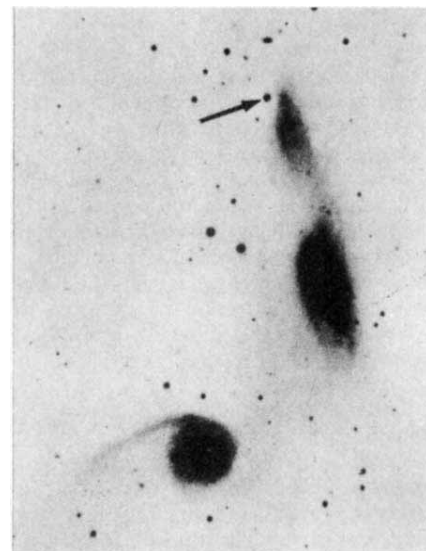
BL LAC OBJECTS

Link with Galaxies?

by our Cosmology Correspondent

BL LACERTAE is the archetype of a class of interesting extragalactic objects. These BL Lac objects seem to be exceedingly compact, and are in many ways reminiscent of QSOs, with the important difference that they have no spectral lines and thus no measured redshifts. Although only half a dozen of the objects are known, and perhaps no more than three can be confidently said to be of the same family as BL Lac itself, they have recently been the subject of intensive study because they may be related to QSOs.

One interesting speculation—it is no more at present—is that BL Lac objects may be blueshifted QSOs; their flat,



NGC 2993 (bottom), NGC 2992, and Weedman 2 (arrow). (Plate obtained by H. C. Arp.)

line-free spectra could well represent a region "normally" seen in the infrared. This idea is important, even though the sample of BL Lac objects is so small, because the definite detection of even one blueshifted QSO would have a significant bearing on the "redshift/distance relation" of QSOs. There is mounting evidence that the redshift of QSOs cannot be explained simply as a Doppler effect of motion in an expanding universe, and that part of the redshift, in some QSOs at least, must arise in some other way.

Paramount among the reasons underlying this view is the discovery of some

QSOs which seem to be physically associated with galaxies. In some such associations, the QSO has a redshift much larger than that of the galaxy with which it is associated, although such an association would imply that both objects are at essentially the same cosmological distance and should thus, on a simple cosmological model, be receding at the same velocity.

Interest in this situation is now heightened by the discovery of a blue stellar object which is probably a member of the BL Lac family and seems to be associated with the galaxy NGC 2992 (Burbidge *et al.*, *Astrophys. J. Lett.*, **178**, L43; 1972). NGC 2992 has already been noted as part of an unusual system; Burbidge *et al.* reproduce the illustration from Arp's *Atlas of Peculiar Galaxies* (see figure) which shows the distortion of this galaxy and its neighbour NGC 2993, and the bridges of luminous material which not only seem to link the two galaxies but also extend in the direction of Weedman 2, a BL Lac type object. As Burbidge *et al.* point out, "If a physical connection could be established between Weedman 2 and NGC 2992, it would be of great importance in establishing the nature of the QSOs and/or the BL Lacertae type of object".

Like the other eight very blue stellar objects appearing near galaxies which were catalogued by Weedman (*Astrophys. Lett.*, **9**, 49; 1971), No. 2 was chosen because it is blue (the spectrum shows an ultraviolet excess), brighter than 19 mag on the blue plates of the Palomar Sky Survey, and close to a galaxy with a maximum diameter greater than 1 arc min. Weedman 2 is the only object which this search found near to one of Arp's peculiar galaxies; it is also interesting that Weedman's criteria are so restrictive that many known QSOs would not qualify for selection under them.

Burbidge *et al.* have found that the continuous spectrum of Weedman 2 is very similar to that of many QSOs. Short of 6,000 Å, there is a slow rise towards higher frequency, roughly a power law with index ~ 0.3 ; at longer wavelengths there is a steep negative slope, index ~ -4 . There are, however, no discrete spectral features. Weedman 2 is, then, a QSO without spectral lines, or a BL Lac object. Both the nearby galaxies are highly excited systems, showing strong emission in the Balmer lines, some oxygen lines, and other features. They have much the same redshift. Is Weedman 2 physically associated with NGC 2992?

If all three objects are at the distance implied by a straightforward cosmological interpretation of the galaxies' redshift, then the distance from Weedman 2 to the centre of NGC 2992 is only 22 kpc, roughly the same as the diameter

of our Galaxy. There is no doubt that gaseous material could extend out to this distance from NGC 2992, and the luminous matter seen in the direction of Weedman 2 might well be being excited by radiation from that object to make it the prominent feature visible on the plates. The energy which Weedman 2 must be emitting, if at the distance implied by the redshift of NGC 2992, is 5×10^{42} erg s⁻¹, typical for a bright spiral and so both adequate to account for the radiation from the luminous bridge and small enough to be plausible.

GLACIATION

Argentinian Chronology

from our Geomagnetism Correspondent

ALTHOUGH the delineation of the geomagnetic polarity-time scale for the past few million years has played an important part in defining global concepts and processes, geologists and geophysicists have recognized for many years that in the longer term the scale should be no less useful in elucidating more local stratigraphic problems. This is likely to be the case particularly where radiometric dating is not possible or where precision in dating is difficult to achieve, although even where accurate

radiometric dating is technically feasible the use of palaeomagnetic techniques may be quicker and cheaper, especially where there is some advantage to be gained from the study of many individual rock units. Even in cases where the statistical problem is less acute, however, there may still be some benefit in employing palaeomagnetism, whether or not independent radiometric ages are available.

A good example of the combined use of palaeomagnetic and radiometric dating is provided by a recent study of late Pliocene and early Pleistocene glaciation in southern Argentina, carried out by Fleck *et al.* (*Earth Planet. Sci. Lett.*, **16**, 15; 1972). The site investigated was on the Cerro del Fraile, a hill about 200 km south of Lago Argentina which comprises Cretaceous sediments capped unconformably on its western face by about 180 m of late Cainozoic basalt lava flows with interbedded glacial and fluvial deposits. The stratigraphic sequences studied by Fleck and his colleagues are more adequately represented pictorially but can simply be described as (starting at the base) cobbles-sand-1-A-cobbles-B-2-C-3-D-4-E (not sampled)-5-F-G-6-H, where the letters refer to basalt lava flows and the numbers to till layers.

Huntite makes a Brighter White

IN next Monday's *Nature Physical Science* (January 8), Veen and Arndt describe for the first time the occurrence in soils of the mineral huntite, CaMg₂(CO₃)₄. But huntite has an alias, pahn-jahn, and this makes their report of more than mineralogical interest alone.

Huntite was first described about twenty years ago from magnesian limestones in Nevada. Since then it has been found in evaporites along the Persian gulf and in secondary carbonate deposits in limestone areas. Now it has turned up in some Australian soils.

The soils in question are vertisols, or cracking clay soils, formed over basalts near Katherine, Northern Territory. Vertisols are almost exclusively found in those parts of the tropics with a marked dry season. Their name derives from the heaving of the soil layers, caused by the presence of clay minerals like montmorillonite which swell on wetting.

During the dry season there is an upward movement of moisture caused by evaporation losses from the ground surface. As the result of the concentration of dissolved salts by evaporation, carbonates, sulphates or chlorides of Ca, Mg, Na and K may be precipitated, the relative amounts of the various ions determining what minerals form.

In the case considered by Veen and

Arndt, the dominant mineral would be expected to be dolomite, CaMg(CO₃)₂. But, perhaps because the basalts are rich in magnesium, huntite and aragonite (a variety of CaCO₃ not often found in soils) have formed instead. Veen and Arndt think that the explanation lies in the ability of small amounts of Mg ion to inhibit the formation of calcite layers, common to both dolomite and calcite crystals. The amount of Mg²⁺ present is, however, insufficient to allow the formation of magnesite (MgCO₃), and under these conditions the stable carbonates are huntite and aragonite.

Alias pahn-jahn, huntite powder is a white pigment which the Aborigines prefer to kaolin-based paint for personal and other decorative purposes. The reason for this preference, Veen and Arndt suggest, is the greater lustre and reflectivity of carbonates as compared with clay minerals generally. In the particular case of huntite, the effect may be enhanced by the occurrence of the mineral as minute but perfectly oriented crystals.

Assuming that awareness of the colouring properties of pahn-jahn dates from the distant past, one might infer that discovery honours for the mineral in soils should really go to the Aborigines. But at the very least, Veen and Arndt have now provided a chemical gloss for the term.

The whole sequence overlies the Cretaceous sediment.

Potassium-argon ages ranged from 2.06×10^6 yr for flow B to 1.03×10^6 yr for flow H, flow E not having been measured and the age of flow A being intermediate. All the flows (certainly B-H and probably A as well) were thus extruded during the Matuyama reversed geomagnetic epoch. But whereas flows A, D and G were reversely magnetized, flows B, C, F and H were normally magnetized. It is clear from these relationships, therefore, that three separate normal events within the Matuyama epoch had been sampled.

Specific radiometric ages (2.06×10^6 yr for flow B, 2.05×10^6 yr for C, 1.67×10^6 yr for F and 1.03×10^6 yr for H) suggest these to be the three known principal normal events—the Réunion, the Olduvai and the Jaramillo—although there are some small discrepancies between these results and previously accepted values. A new summary by Dalrymple (in *Calibration of Hominoid Evolution*, Scottish Academic Press, Edinburgh, in the press), for example, shows the split Réunion event to be represented by normal flows in the age ranges 1.95 to 2.01×10^6 yr and 2.08 to 2.09×10^6 yr. The ages of flows B and C could thus imply either that at least one of these ranges requires extension or that the Réunion event is really a triplet. Moreover, the Jaramillo event is usually said to have begun about 0.95×10^6 yr ago. The age of flow H thus implies either a Jaramillo event starting rather earlier than hitherto supposed or a split event. These small differences apart, however, it is clear that the Cerro del Fraile section includes all three principal normal events in the Matuyama epoch; and in this respect it is unusual if not unique.

The existence of six till layers certainly indicates repeated glaciation and, assuming none of the lava flows to be subglacial, would seem to imply no less than six glacial events. Geological and petrological evidence previously adduced by Mercer (*Science*, **164**, 823; 1969), while not ruling out a subglacial origin for every single flow, nevertheless militates against such an origin. Thick baked zones cover several of the tills, for example, and no hyaloclastic material was detected in any of the samples examined petrologically. Moreover, the fact that individual flows cover a distance of up to 4 km suggests free flow over a gently sloping surface. Further evidence for at least one non-glacial period between glacial events represented by till is the existence of fluvial cobbles and gravel between flows A and B.

The age relationships between glacial and non-glacial periods are deduced by reference to the palaeomagnetic-radiometric time sequence. The earliest

glaciation is represented by the till (1) beneath flow A. The age of flow A is not precisely known but the flow must be older than the 2.06×10^6 yr of flow B and, because it is reversely magnetized, presumably younger than the 2.43×10^6 yr age of the Matuyama-Gauss boundary. The second glaciation, which follows the ice-free period represented by the cobbles above A, is precisely dated by the till (2) which lies between the 2.06×10^6 yr-old flow B and the 2.05×10^6 yr-old flow C. The third glaciation (till 3) occurred less than 2.05×10^6 yr ago but earlier than the 1.86×10^6 yr represented by the non-subglacial flow D which indicates another ice-free period. The area was then glaciated twice (tills 4 and 5) between 1.86 and 1.67×10^6 yr ago and at least once (till 6) between 1.47 and 1.03×10^6 yr. There is no evidence of glaciation between flows F and G dated at 1.67 and 1.47×10^6 yr, respectively.

Finally, what, Fleck and his colleagues ask, are the climatic implications of these repeated glaciations? At present, the Cerro del Fraile, being in the rain shadow of the Cordillera, has no permanent ice cover. Nevertheless, if the landscape were less dissected between 1 and 2×10^6 yr ago, as it must

have been, the Cordilleran ice could have covered the Cerro del Fraile even under climatic conditions similar to those of today. Certainly there seems to be no necessity to invoke lower temperatures to explain the repeated glaciation although, in view of the lack of information on ancient elevations, neither can lower temperatures be ruled out *a priori*.

Unfortunately, as Fleck *et al.* point out, existing southern hemisphere palaeotemperature data (from marine strata now exposed on land or from microfossil and coarse-sediment content of deep sea cores) are not conclusive on this point because of disagreement between the workers involved. Nevertheless, Cerro del Fraile glaciations come to the closest agreement with the palaeotemperature scheme of Kennett *et al.* (*Science*, **171**, 276; 1971) who proposed coolings at 2.5×10^6 yr ago, between 2.13 and 2.10, between 1.98 and 1.88 and between 1.77 and 1.61×10^6 yr ago. Other schemes are also consistent with the Cerro del Fraile data, if less so; but some—for example, the warmer climate between 1.8 and 1.6×10^6 yr suggested by Bandy *et al.* (in *Antarctic Oceanology*, **1**, American Geophysical Union, 1971)—are certainly not.

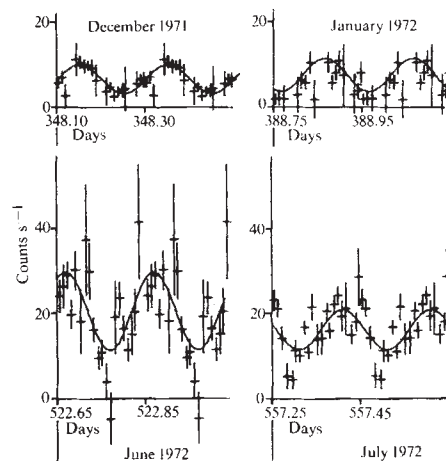
Variations of Cygnus X-3

By combining data obtained from three satellites, Canizares *et al.* have been able to deduce a very accurate period for the X-ray variation of Cyg X-3. This source has been observed by the Uhuru and Copernicus satellites, and both studies have revealed a variation in the X-ray flux with a period of approximately 4.8 h. Since the recent radio outbursts from the source (see *Nature Physical Science*, October 23, 1972) Cyg X-3 has been the subject of many searching investigations; in next Monday's *Nature Physical Science* (January 8) a team from MIT report observations made by OSO-7 in December 1971 and in January, June and July 1972. From these observations and those made by the two other satellites, a clearer picture of the behaviour of Cyg X-3 is now emerging.

The most notable feature, apart from the regular variation, is that the mean intensity of the source is not constant. Between December 1971 and June 1972, the mean changed by more than a factor of three. Also, the shape of the best-fit curve to the data points changed over a six month period, so that in the later observations the changes from maximum to minimum intensity are more abrupt than they were in December 1971 and January 1972. There are also variations in the intensity of Cyg X-3 and these show no regular pattern but occur on a timescale of tens

of minutes, reminiscent of the variations seen in other discrete X-ray sources.

If the period of the source is assumed constant throughout the period of observation by the three satellites, which extends from December 1971 (OSO-7) to September 1972 (Copernicus), it is fitted best by a 0.199672 ± 0.000008 day variation. The OSO-7 data alone suggest a period of 0.199667 ± 0.000014 day, and the Copernicus data alone give 0.1997 ± 0.0004 day. Canizares *et al.* suggest that the data imply a period constant to 1 part in 1,500 over 7 months.



OSO-7 data for Cyg X-3 with best fit curves. Error bars show uncertainties of one sigma.

Monitoring Underground Explosions

DAVID DAVIES

Lincoln Laboratory, Massachusetts Institute of Technology, Lexington, Massachusetts 02173

Seismological means for detecting and identifying underground nuclear explosions have improved steadily during the past ten years. A technique exists for separating explosions from earthquakes. The problem now is lowering the threshold and understanding the occasional problematic event.

AN underground nuclear explosion converts about 1 per cent of its energy into seismic waves and these carry information about the time, size, and location of the event. They may also indicate that the event is indeed an explosion and not an earthquake. This information is somewhat more difficult to extract, and research in many countries for the past few years has been intensively directed towards discrimination between natural and artificial events. There is no other known way of identifying underground explosions that can be used on a world-wide basis. In this article, I shall describe the progress that has been made recently in this science, which has such obvious implications for arms control. The conclusions that I reach should not, however, be construed as a measure of the prospect of a total test ban. Many ingredients go into the making of a treaty, and ability to police it is only one of them. Seismological capability is thus not sufficient, but it may be necessary, at least down to some level of explosive yield. What would constitute an adequate level is a matter of some discussion at present. The science reported here is the result of the research of many seismologists. In order to reduce the number of references to a manageable level, however, I have cited only a few large compilations of results in which the genealogy of the various ideas and instruments may be found.

The reason for testing nuclear weapons underground is not, of course a matter of public discussion, but a paper by Neild and Ruina¹ probably provides a reasonably complete list of purposes. Between 1968 and 1971 there was an average of about twenty-five underground tests a year announced by the United States compared with about ten presumed tests a year for the Soviet Union, one test per year for China and about five a year for France—all but one of the Chinese tests and all of the French tests being atmospheric. Britain has not announced a test for seven years.

When the banning of nuclear tests first became an international issue in 1958, a conference on the technical problems in Geneva clearly indicated that the science of seismology would need substantial advances to reach a stage at which instrumental observations could indicate unambiguously that an explosion with a yield of a few kilotons (kton) had been detonated. Indeed the rather meagre data available during that conference and the subsequent negotiations (there had at that time only been two or three quite

small underground tests) suggested that a first objective could reasonably be an international network of about 170 stations which could detect seismic signals down to a certain threshold but not necessarily identify their source. It was expected that most seismic events would clearly indicate their earthquake nature by their location and depth, their radiation pattern and the shape of their signal, but there would be a residue which would need further investigation. In the early days of international negotiations, inspections of the sites of a fraction of these suspicious events were discussed in detail—the number and nature of such inspections being particularly contentious topics. With time, the position of the Soviet Union on the inspection issue hardened to the assertion that inspections were unnecessary, and that purely national means of policing would be satisfactory. Thus the "Geneva network" was never built.

The two issues which were seen as central in 1958 are central today. Background seismic noise (from wind, traffic, ocean waves, and so on) placed a limitation on the detection of events; and a certain number of earthquakes did not immediately reveal themselves as such. The advances in seismology have been substantial since 1958, but increasing the signal-to-noise ratio (s.n.r.) and finding improved methods of separating earthquakes and explosions are still the principal fields of research. The rather narrow frequency bands involved in seismology have controlled techniques rigorously, and because the work is concentrated in situations of low s.n.r., the problem has been that of finding the frequency at which the s.n.r. is highest, and aiming improvement at that frequency, rather than trying to encompass the whole spectrum of the seismic signal. This "mission-oriented" approach to seismology, when combined with the intellectually stimulating problems that have arisen on the way, has exerted a vital role in the development of seismology since 1958.

It was clear from very early in the Geneva discussions (which lasted until 1962) that whatever international arrangement might finally develop, national interests required an improved seismic capability. The United States, Britain and Sweden, in particular, started to modernize their seismological capability for the explicit purpose of test-ban monitoring. Other countries, notably Canada and Japan, were also improving their seismic capability. The US programme "Vela Uniform" has been easily the largest, and so far more than \$150 million has been spent on research and development. What has been bought?

Recording of Seismic Signals

In the first few years basic research was heavily supported and a network of 100 seismic stations, the World-Wide Standard Seismographic Network (WWSSN), was established—no stations were, however, sited on the territory of the Soviet Union or her allies. The network provided a huge data base, uniformly recorded, of short period (around 1 s) and long period (around 20 s) seismic signals.

Certain "realities" soon emerged from this work. The

decreasing prospects of an international network with access to the Soviet Union led to the dominance of teleseismic studies; seismic body waves (P and S waves) are well registered up to a few degrees from the source; indifferently and variably from there to about 25° ; and then clearly and predictably out to 100° . Distances are measured in central angle degrees. Some examples of P waves are given in Fig. 1. This last, teleseismic, zone covers half the world and would be crucial if access were not permitted to particular countries. The zone of observation having been restricted, immediate limits can be placed on the detectability of explosions. A 5 kton explosion in hard rock, such as granite or salt, produces in the teleseismic zone a P wave of amplitude around 5 nm at a period of 1 s. Background noise at 1 s is about a third of this figure for the best observatories. Thus in the absence of techniques for noise rejection, a network would usually detect a 5 kton explosion in hard rock (an event cannot be said to have been detected until recorded with a signal-to-noise ratio of at least 1.5 at four widely spaced stations). The capability declines drastically below this yield. Further, explosions in softer rocks, notably tuff, a volcanic ash common in Nevada, produce a smaller signal by a factor of at least two than comparable explosions in hard rocks. Explosions in dry alluvium have even smaller seismic effects—1 kton in hard rock and 10 kton in alluvium are roughly equivalent. There is, however, a limit to the thickness of dry alluvium to be found anywhere on Earth, and as an explosion has to be adequately buried for safety's sake, it is unlikely that explosions of more than about 20 kton can be fired and contained in known deposits of alluvium.

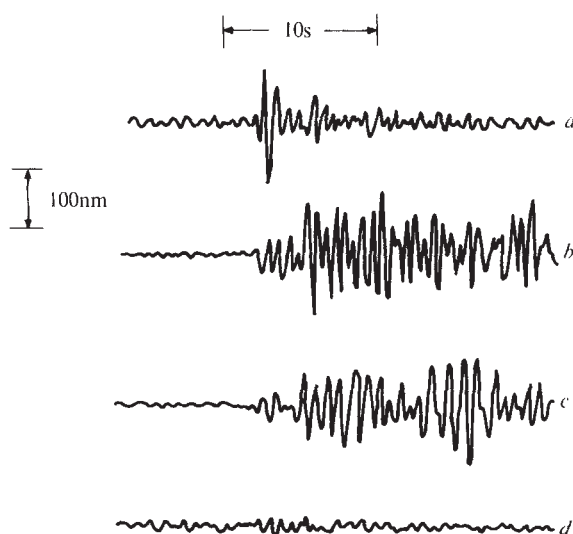


Fig. 1 Short period teleseismic observations of P waves from events located in *a*, Eastern Kazakh, USSR; *b*, Alaska; *c*, Turkey and *d*, South Sinkiang Province, China. The top and bottom events are presumed underground explosions. All events have body wave magnitudes in the range 5.0 to 5.7. Note the great difficulty there would be in identifying the direction of first motion for these events.

At the same time that the WWSSN was being equipped with conventional instruments and photographic recording, several experiments with arrays were being conducted. The aim was to suppress noise, presumed incoherent, by the addition of many channels. Another advantage accrued in the recording of data on analogue and later digital tape. Although practically all decisions in seismology are taken on the basis of observation by eye, direct visual recording suffers obvious disadvantages of dynamic range. Further,

there is a strong noise peak at a period of about 6 s arising from microseisms generated in the oceans and the rejection of these by WWSSN instruments, peaked at 1 s and 20 s, is usually not entirely satisfactory. As a result, film records, in which the magnification is controlled by the ease of viewing, are often dominated by 6 s microseisms and are thus not running at the best magnification for 1 or 20 s signals. The data processing possibilities of tape recording remove this obstacle.

The first arrays built in the United States had an aperture of 4 km. They were in many ways prototypes of possible arrays that the Geneva meetings had envisaged for an international network. Up to sixteen seismometers were spread out over this aperture, and the signals from all seismometers were added without phasing. Seismic signals from teleseismic distances arrive at steep angles—the signal sweeps across the ground with a horizontal phase velocity of at least 10 km s^{-1} and up to 24 km s^{-1} , and at typical sites this corresponds to angles of incidence of less than 20° . Thus straight addition does not seriously degrade the 1 s component of a signal seen in the small aperture. It was found with these small arrays that the noise was coherent in this frequency band for distances typically greater than the 0.5 km spacing of instruments, so the gain in s.n.r. was somewhere between one (if the noise were totally coherent and travelling in a near vertical direction) and $\sqrt{N}$ (if the noise were totally incoherent). Later arrays have used a seismometer spacing of around 2 km, at which distance local noise is substantially incoherent.

The next development was the medium aperture phased array implemented by the United Kingdom Atomic Energy Authority². Up to twenty seismometers were laid out in two perpendicular lines. Arrays of this type are in operation in Canada, Brazil, Scotland, India and Australia. The aperture was large enough to require phasing of the signal after recording to steer the array in the appropriate direction, but small enough to ensure that the signal was still coherent. Signal-to-noise improvements approaching $\sqrt{N}$ (about 4.5) are obtainable.

The first large aperture seismic array (LASA in Montana) was completed by the United States in 1965, and a second array (NORSAR in Norway) has been in operation about two years. LASA has an aperture of 200 km and 350 short period seismometers; NORSAR is somewhat smaller. LASA is steered (by a computer) to be face-on to incoming seismic signals and the angle of approach is sufficiently well determined to locate the source with an accuracy of one or two hundred km. The real time digital operations of LASA and NORSAR each occupy entirely two medium sized computers in hunting for P waves. The gain for large arrays does not meet the $\sqrt{N}$ expectation, however, for over an aperture of 200 km crustal geological conditions can vary widely and this leads to a degrading of the coherence of the signal. An improvement in s.n.r. of a factor of ten is possible for LASA, however, and the array is capable of detecting and locating explosions in the teleseismic zone with yields down to about 2 kton in hard rock. Several thousand earthquakes occur each year with P waves of comparable or greater amplitude, and these form the population against which explosions have to be discriminated.

It became increasingly apparent in the mid 1960s that the registration of body waves was not sufficient to solve the discrimination problem; a broader spectrum of seismic data was necessary. The answer lay in the dominant signal on the long period seismic traces, the Rayleigh wave. This is a dispersed wave which has travelled over the Earth's surface and is well excited at 20 s periods—there are short period Rayleigh waves and long period P waves, but they are unimportant for discrimination. As for short period recording, noise is the limiting factor. The 6 s oceanic microseismic background, longer period fluctuations arising

from atmospheric effects and the interference of Rayleigh wave trains from other earthquakes all contribute to the background. In the absence of an interfering event, most seismic stations have a background noise level at 20 s period of 50 to 100 nm; there is a great variability in noise level both from place to place and in time. For the reasons already given, tape recording is most desirable.

Rayleigh waves spread cylindrically, but also undergo other attenuation in the ground. The signal decays as distance (Δ) to the power 1.6. This rapid decay makes it imperative that the seismic station be sited as near to the event as possible. Within a political requirement of non-intrusive monitoring we have to consider a value of Δ of 20° as the minimum at which stations will be available. At 20°, the Rayleigh waves from a 10 kton explosion with 20 s period are somewhat less than 100 nm in amplitude, so individual stations at this distance have a relatively small probability of detecting surface waves. As with short period observations, arrays prove a valuable means of increasing the s.n.r. Instrument spacings have to be greater (typically 20 km) for noise to be incoherent. Small tripartite arrays are either under construction or recently completed in Sweden, Canada and India. LASA, NORSAR and ALPA (an exclusively long period array in Alaska) all have at least fifteen long period seismometers within them. Results from LASA indicate an improvement in s.n.r. of a factor of four—close to $\sqrt{N}$. In addition, knowledge of the nature of the dispersed waveform allows matched filter techniques to be applied, and these contribute up to another factor of two to the signal-to-noise ratio. Thus a twenty element long period array can yield an order of magnitude improvement in the s.n.r. against incoherent noise. In addition an array has a certain amount of directional discrimination (Rayleigh waves have a wavelength of 80 km at 20 s period) which makes it possible to study an event in the presence of an interfering signal, provided that the two are coming from directions differing by more than 30° and that the interfering signal is no more than a factor of ten larger than the signal being studied.

Recent developments in instrumentation have been successfully aimed at reducing the non-seismic noise in instruments by more effectively sealing and isolating them, and at broadening their response to cover a wide spectrum. There is also promise in the siting of instruments down boreholes where long period noise is frequently substantially lower.

Seismic Magnitude

Both P waves and Rayleigh waves are used to define a magnitude for seismic events. The body wave magnitude (m_b) on the Richter scale is a measure of the excitation of P waves. The amplitude of the P wave signal on the record is converted to ground displacement (A) at a dominant period T , and then used in a formula

$$m_b = \log_{10} \frac{A}{T} + B(\Delta)$$

where $B(\Delta)$ is a term counteracting the variation of the signal with distance from the source. A ground displacement of 10 nm at 1 s period at a distance of 60° from a source indicates an event of magnitude 4.7, for example.

The surface wave magnitude is obtained by measuring on the Rayleigh wave the ground displacement A at a period T (usually near 20 s) and using a formula

$$M_s = \log_{10} \frac{A}{T} + 1.66 \log_{10} \Delta + 0.3$$

An event with an M_s of 4.0 will have a ground displacement at 30° of 300 nm at 20 s period.

For earthquakes, there is an empirical and very scattered

relationship between M_s and m_b , the best fit to which is

$$M_s = 1.59m_b - 3.9$$

I shall discuss this relation further later.

It is possible to relate both m_b and M_s to the yield for explosions. Two kton in hard rock produces an event with an m_b of 4.0, 20 kton an event with an m_b of 5.0. For higher yields the equivalence of a factor of 10 in yield to a change of one unit in magnitude breaks down for several reasons. I have already noted that softer rocks produce smaller seismic signals; an m_b of 4.0 is equivalent to 2 kton in granite, 3 or 4 kton in tuff and 20 kton in alluvium.

The surface wave magnitude M_s can equally well be related to the yield, and is a more useful measure, for it seems to obey a relation (for hard rock) of the form

$$M_s = 1.3 \log_{10} Y + 1.5$$

over a range from at least 1 kton to 1 mton.

Discrimination

In the early development of the subject, the concentration on the detection of events by means of P waves led to a hope that the study of P waves alone might show differences between explosions and earthquakes. It was soon found that such differences did exist, but not to the extent that all earthquakes could be separated from all explosions; it was only possible to quote probabilities. Diagnostic aids, as they have come to be called, are the location and depth of the event, the direction of first motion of the seismic trace and the complexity of the signal. Clearly if an event can be unequivocally identified as having occurred deeper than a few km it is not an explosion—likewise if it occurs under deep water. The quality of depth determination declines as the depth decreases and it is frequently impossible to assign depths to events shallower than 30 km. The first motion criterion uses the difference in radiation pattern between a totally compressive explosion and an earthquake for which the radiation is successively compressive and rarefactional in adjacent quadrants. There are numerous problems—the uneven distribution of stations around the globe and the high signal-to-noise ratio required to make polarity measurements both render the technique of little use for lower yields. “Complexity”, a measure of the duration of the P wave signal, is a criterion which showed promise; signals that last more than a few seconds are likely to have come from earthquakes. Unfortunately there are many earthquakes which last for less than a second and these must be placed in the category of “suspicious events”.

More recent work on the spectra of P waves has shown that even explosions of very small magnitude are significantly richer in high frequencies (2 to 3 Hz) than are earthquakes. This accords with generally accepted ideas of the nature of earthquake and explosion sources. Regional variations present problems, however; it seems at present that the attenuation (and hence the frequency transmission characteristics) of the Upper Mantle varies sufficiently from place to place that genuine spectral differences between earthquakes and explosions are overprinted with the frequency absorption of the Upper Mantle in such a way that P waves from explosions can occasionally look like those from an earthquake.

The broadening of the spectrum that the joint study of Rayleigh and P waves brought with it provided the one indubitable “breakthrough” that the subject has seen. It had been realized for several years that explosions did not generate anything like the amount of Rayleigh waves that did earthquakes of comparable body wave magnitude. The WWSSN allowed these observations to be put on a more quantitative and global basis. It was noted that if the

surface wave magnitude M_s for each event was plotted against its body wave magnitude m_b , the natural and man-made events clustered round two well separated lines.

A typical example is shown in Fig. 2. Clearly the earthquake population has a wider scatter than the explosion population, and this is unsurprising considering the diversity of earthquake types. Vagaries of propagation in the real Earth also contribute to the scatter. Nevertheless one point can be asserted—that the diagram has greatly increased our confidence in identifying events above a certain size. Of course the purist may reasonably object that a diagram such as Fig. 2 cannot offer certainty as it is only a modest sized population; nevertheless the diagram has been widely accepted.

Earthquakes and explosions generate other seismic phases—shear or S waves and horizontally polarized surface waves called Love waves. In addition P waves have a long period component. All of these offer possibilities for discrimination, particularly because explosions in theory generate few S and Love waves. For large explosions and earthquakes, use of these waves works well, but it is found in general that the largest wave amplitude on the long period record is that of the Rayleigh wave.

$M_s : m_b$ Discriminant

Fig. 2 is taken from a recent study³ using WWSSN instruments only. Each point represents one event in Eurasia and it is necessary to have P wave detections at at least four widely separated stations before an event is listed. This places a lower limit on the body-wave magnitude for which events are reported. There are several reasons why this threshold should be rather blurred, but as a gross simplification one can say that the global network of seismometers (excluding arrays) will detect by P waves at four or more stations almost all events with an m_b of 5.0 and greater and almost no events with m_b less than 4.0.

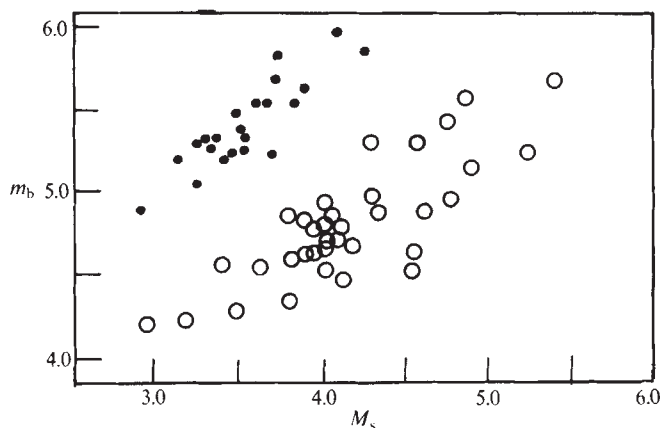


Fig. 2 An $M_s : m_b$ diagram for Eurasia based on WWSSN recordings by Marshall and Basham. ○, Earthquakes; ●, presumed explosions.

Each point also represents a determination of surface wave magnitude, and it is customary to require at least four stations to have detected the surface wave before a value is accepted (this is a very crude way of allowing for the radiation pattern of earthquakes). An event with an M_s of 4.0 is certain to be detected by its Rayleigh waves, but an event with an M_s of 3.0 is unlikely to be detected. This is partially a statement about noise, but the locations of the receivers are also important as they need to be as close to the event as possible.

The thresholds of detection are such that an earthquake is likely to be seen both by its P and Rayleigh waves or by neither. This means that there are few earthquakes which

are assigned an m_b but cannot be placed on the diagram because M_s is not measurable. On the other hand, there may be explosions which have, for instance, an m_b of 4.5 and (by extrapolation) an M_s of 2.5. If it is required that an event may be identified only if both parameters can be determined one can thus talk about a threshold for discrimination as the Rayleigh wave detection threshold. This threshold is statistical in nature, but may be defined in some such way as "If an event detected by P waves has an M_s of 3.2 or greater there is a 90 per cent chance that its surface waves will be detected at four or more stations and thus that it can be categorized as an explosion or earthquake". This somewhat portly statement is necessary because events are routinely detected by body waves but the crucial detection for discrimination purposes is of surface waves. An alternative version is "Explosions in hard rock of 20 kton or more can be identified as such; so can practically all earthquakes detected by the WWSSN".

It is obvious from Fig. 2 that any attempt to lower the yield threshold should reasonably start from an attempt to improve the detection of the surface waves, for it is demonstrable that there are events in Russia with an m_b of less than 5.0 which have no surface waves visible on present instruments but which may be presumed to be explosions. Obviously as a programme for improvement of surface wave capability developed it would be highly desirable to boost up body wave detection in order that almost all events with m_b greater than 4.0 be reported. Such a strategy is discussed later.

Science of Discrimination

Fig. 2 clearly indicates a difference in excitation between 20 s Rayleigh waves and 1 s P waves for the two types of event. A natural question is how this difference arises, and the question is not purely academic, because in attempting to lower the threshold (that is to bring in points with lower M_s and m_b) it is valuable to know exactly when the two populations merge. In Fig. 2 there is a clear separation, but least squares straight lines may not be the best representations of the relationships at lower magnitudes. Theoretical models for earthquakes and explosions should be extendable to any magnitude and thus should indicate whether capital investment to improve detection capability would be justified.

Unfortunately knowledge of the nature of both types of event is still shadowy. An earthquake is known to be a propagating rupture on a fault plane that leads to a finite offset across the fault; and an explosion as seen by seismometers is equivalent to the sudden application of an elastic stress on a notional surface of radius less than 1 km. It is also known in part how P waves and Rayleigh waves are generated from both types of source. This turns out not to be sufficient to answer the question of why the discriminant works. The question can be subdivided by recognizing that the discriminant may depend on the different partitioning of energy at source into body and surface waves; or differences in spectra at source that are reflected in differences in excitation at 1 and 20 s periods regardless of wave type; or some combination of these two.

I do not believe that we yet have a simple answer to this issue. This reflects the inability as yet to describe satisfactorily the processes at work at the focus of an earthquake. I think it is reasonable to say that it is not a depth dependence nor a size difference that controls discriminative capability. Beyond this it is difficult to go.

A more direct and at present more successful approach to the problem of the smaller magnitude events which are as yet beyond the global network's accessibility is to do pilot work in a region where there is the opportunity to get close in to events. Accordingly much research has been concentrated on observations of explosions at the Nevada test site

and earthquakes in the western United States. Results have been reported by Evernden⁴ and, even using standard definitions of M_s and m_b , separation is good down to the lowest yields observed. When careful allowance is made for regional propagation effects separation can probably be improved.

These results show that, within the context of relatively unrestricted access to the area, explosions and earthquakes in the western United States can be discriminated to substantially lower levels than the global threshold of 20 kton in hard rock. There seems to be an order of magnitude of improvement possible without encountering insuperable problems of overlap. It is of the utmost importance that this be understood in context. Monitoring of the Soviet Union, for instance, would involve forgoing the nearby stations and attempting to make up for the loss by high grade instrumentation, arrays and digital processing. Further, there is no guarantee that earthquakes and seismic wave propagation in the Soviet Union are comparable to those in the western United States (although there are grounds for optimism). Lowering the threshold of the monitoring network could, however, reasonably be expected to yield a lower discrimination threshold, and a large investment in such an improvement would be justifiable on scientific grounds.

The Future

Several instrumental developments and deployments are under way at the moment. A small number of high quality, long period seismometers are now set up (in Spain, Israel, Thailand, Australia, Alaska and New Jersey). Others, suitable for deep boreholes where noise may be substantially reduced, are in production. Two large new long period arrays, in Alaska and Norway, and three three-element long period arrays in Canada, Sweden, and India, are now operating. The hope is that within two years there will be sufficient data from these instruments to assess a new level of discrimination. It seems reasonable to expect at least a diminution of a factor of two, to 10 kton in hard rock, on the basis of these investments. This is equivalent to saying that in monitoring the Soviet Union almost all events with an M_s of 2.8 or greater will show detectable surface waves at four or more widely spaced stations. As a goal this is quite modest; the two large arrays plus the new stations in Thailand and Israel alone should accomplish this.

A considerably lower threshold seems, however, to be attainable. Extrapolations from the western United States suggest that it is not at all unreasonable to expect discrimination to be feasible at an M_s of 2.0 (2 to 3 kton in hard rock). How would this be achieved? If a monitoring network is allowed the luxury of expanding, the country being monitored must be allowed the luxury of moving its test-site to the most remote spot possible. It would be possible for the Soviet Union to test at a distance of 30° from the nearest seismometers in a monitoring network, and, at this distance, surface waves from an explosion with an M_s of 2.0 are less than 10 nm peak to peak. There are few stations with a noise level as little as a factor of ten higher than this; and a factor of ten might be the upper bound on what the largest possible array might contribute to the improvement of signal-to-noise ratios.

At this stage, economics may control the threshold more than seismology. For instance, Professor J. N. Brune of the University of California, San Diego, recently estimated that a thorough programme to upgrade seismic capabilities to such a high level would cost at least \$200 million.

How might a major programme reasonably develop? The present operation of WWSSN is decidedly non-optimal, as the network was not designed for, nor is it dedicated to, discrimination. Resiting of some seismometers, particularly removal from centres of population, would yield reduced

noise levels. Better protection from environmental changes, particularly pressure and temperature fluctuation, could not but be beneficial. The aim should be to record ground noise only, and it is worth a substantial amount of time and money to ensure that this is all that is coming out of the instruments. Placing some instruments down boreholes could certainly reduce noise further. Digital recording, in parallel with photographic recording, is highly desirable, preferably with prefiltering to eliminate 6 s microseisms.

At present the WWSSN produces data, but no one is required to analyse them. A small group of seismic analysts, having available both the superior data and also information from the large arrays and other seismic stations that cared to contribute, would greatly increase our number of body-wave detections of events and would develop knowledge about which stations were most useful both in body and surface wave observations. This knowledge would help decide what the next step should be.

It is likely that certain stations would be worth even further improvement. Spotting these sites involves more than just measuring the noise. Seismology is not a wholly predictable subject and, in some locations, signals are unusually high for little known reasons. This may work in favour of sites which would be rejected on the grounds of noise level alone. The improvement might take the form of development of arrays centred on good stations. The establishment of new stations in favourable locations would also make sense.

Beyond this stage it is not easy to speculate. Once one has started reinstrumenting the globe there is no limit to the ingenuity that could be employed. Political and economic realities, however, restrain the monitoring network from extending too far. The development sketched out might be accomplished within five years.

Obstacles

The relative simplicity of Fig. 2 may give the impression that discrimination can be made into a list of instructions for seismic record readers. In most cases this is true, but there are nagging problems—some natural and some man-made.

In considering a population of events in a certain region it turns out that some events cannot be categorized on the basis of an $M_s:m_b$ discrimination because the surface waves from them are masked by surface waves from other unrelated earthquakes. These cannot be discriminated by the $M_s:m_b$ technique unless a way can be found to separate the two events. An array is highly desirable for this, and even then there are prospects of success only under the circumstances discussed earlier. If the masking event is very large (with an m_b of 7.5 or greater) the global network is blotted out to such an extent that detection of even P waves from practically any event for an hour or more afterwards may be impossible because of the reverberations within the Earth. This opens up the possibility for evasion by firing an explosion immediately after a large earthquake. It is difficult at present to see any seismic means of improving the prospect of detecting such evasions.

Another natural hazard is that of explosion-like earthquakes. If the time interval for which Fig. 2 had been compiled were expanded, earthquakes of quite high magnitude (an m_b of 5.0 for instance) but which produce barely discernible surface waves would eventually be found. These earthquakes, which come from very limited regions on the Earth's surface, would have to be classed as explosions according to the $M_s:m_b$ criterion. It is believed that they can be understood in terms of earthquakes in which the stress drop is abnormally high. Whether with improved instrumentation any earthquake-like features of these events will reveal themselves remains to be seen. It is not yet known whether, with improved body wave detection capa-

bility and hence larger populations of smaller events, more of these earthquakes would be encountered.

The chief man-made problem (apart from deliberate masking by earthquakes) is that of decoupling. The radiated seismic signal from an explosion fired in a sufficiently large hole can be at least a factor of 100 smaller than in the fully tamped case. Thus a 50 kton explosion could be moved below the bounds of detectability if fired in a deep hole of radius 80 m, and partial decoupling would occur if the radius were less than this. Although a removal enterprise of this magnitude might well excite attention if started after a treaty had been signed, there may at present be cavities in salt mines which could be used for partial decoupling. There seems no seismic way of detecting decoupled events, and entrants into a treaty would have to weigh the risks accordingly.

Although there are prospects of reducing the yield threshold to a figure of a few kton in hard rock, it must be borne in mind that a violator is unlikely to fire his explosions in granite. As a threshold of 2 kton in hard rock would be very difficult to achieve, this might be turned into a statement that all tests under 20 kton fired in alluvium would not be discriminable. This depressing situation is fortunately unlikely to be valid. The thickness of alluvium deposits limits the depth of the shot, but, on the other hand, the shot must be buried deep enough not to form a subsidence crater. These craters have formed more often than not after a test in alluvium, and there would be a clear risk of visual detection. Thus a violator would have to judge the risks of alluvium firing and might well conclude that a yield much smaller than 20 kton was all that could safely be tested.

"Negative Evidence"

One may justifiably feel vaguely uneasy about the $M_s:m_b$ discrimination technique. It tends to enshrine a lot of science and technology in one diagram and it may be used misleadingly. For instance although the population of earthquakes in Fig. 2 is fairly representative, there can only be a limited number of explosions; most of these come from one test site and are thus not truly independent samples. Further, test sites could be moved around at will, and so the discriminant can only represent the past, not the future. These qualms can only be assuaged if the science of discrimination is seen to be well founded.

On the other hand, there may also be some uneasiness that the $M_s:m_b$ discriminant is not optimistic enough, and this is worth examining. When an event has both detectable P waves and detectable Rayleigh waves it can be placed on an $M_s:m_b$ diagram. If, however, P waves are detected but no Rayleigh waves are seen, it is unacceptable. For instance, an explosion in the Soviet Union with an m_b of 4.5 will not generate detectable Rayleigh waves. Could it be called an explosion on the basis of its lack of Rayleigh waves? Obviously this question depends on whether all earthquakes with an m_b of 4.5 have detectable Rayleigh waves (we eliminate the masked event problem). The answer is that on present experience they do, with the exception of a limited number of events mentioned earlier. The spread of M_s values for earthquakes with $m_b > 4.5$ is substantial, but it does not extend down to 3.2.

Up to the present so called "negative evidence" has always been looked on with some suspicion. The reason for this is probably that, although to a scientist the absence of a signal can be as important as its presence, to the politician with a different mode of thinking "negative evidence" sounds as unconvincing as would absence of fingerprints to establish not being present at the scene of a crime. It may well be that the $M_s:m_b$ diagram, for all its neatness, has placed an undue emphasis on the necessity for determining both M_s and m_b .

The following would be a "negative evidence" type of discrimination statement. "When an event has been detected by P waves and assigned a body wave magnitude of 4.5 or greater it can be categorized as an earthquake if $M_s \geq (m_b - 1.3)$ and an explosion if $M_s < (m_b - 1.3)$ or if M_s cannot be determined, but on the basis of noise observations must be less than $m_b - 1.3$ ". It would switch the threshold to a body wave magnitude of 4.5 or about 5 kton in hard rock, a figure that with the $M_s:m_b$ discriminant alone would take several years and much expenditure to achieve. Although this statement would let through as explosions those unusual earthquakes described previously, this would equally apply to the $M_s:m_b$ technique. For these events a pragmatic approach is necessary. A very detailed survey with a large population is necessary to establish the general validity of negative evidence. Such work is at present being pursued.

The Broader Scene

I shall conclude with a description of recent activity beyond the laboratory. In 1968 the Stockholm International Peace Research Institute (SIPRI) convened a conference of seismologists from ten countries, east and west, at which technical progress since 1958 was reviewed. Subsequent SIPRI Progress Reports, the latest dated September 1971 (ref. 5), have kept the technical content up to date.

In the United States, numerous technical meetings have been held. Papers presented at the Woods Hole meeting in 1970 (ref. 4) have been widely used in this discussion. A recent meeting in Cambridge, Massachusetts, has allowed some of the projections to be strengthened and a growing appreciation of the problem events.

The Conference of the Committee on Disarmament of the United Nations (which has lived under several names) meets regularly at Geneva, and the issue of seismology and a test ban is frequently aired there. In 1970, at Canadian instigation, a register of seismic stations with guaranteed available data was compiled by the United Nations and subsequently a Canadian survey⁶ examined the theoretical potential of this network. In the same year Britain submitted a proposal⁷ to Geneva on a new network which would improve discriminative capability. In 1971 a United Nations technical meeting was held in Geneva against a background of increased international interest in a test ban.

It is clear that this subject will continue to remain alive before a more general public for the foreseeable future. During this time seismological advances are unlikely to be spectacular, but a continuing investment in seismology will undoubtedly produce a steady reduction of the threshold. Where this threshold ultimately comes to rest and whether a treaty will be signed will be determined by a multiplicity of factors, only one of which is seismology.

This work was sponsored by the Advanced Research Projects Agency of the Department of Defense.

Fig. 2 was kindly supplied by P. W. Basham and P. D. Marshall, and represents work done by them³ at the Department of Energy, Mines and Resources, Ottawa, Canada.

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² *The Detection and Recognition of Underground Explosions* (United Kingdom Atomic Energy Authority, London, 1965).

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Diachronous Deposits: a Kinematic Interpretation of the Post Jurassic Sedimentary Sequence on the Pacific Plate

B. C. HEEZEN

Columbia University, New York

I. D. MacGREGOR

University of California, Davis, California

H. P. FOREMAN

Oberlin College, Oberlin, Ohio

G. FORRISTAL

Shell Development Co., Houston

H. HEKEL

Geological Survey of Queensland, Brisbane, Australia

R. HESSE

McGill University, Montreal, Canada

R. H. HOSKINS

New Zealand Geological Survey, Lower Hutt, New Zealand

E. J. W. JONES

University College London

A. KANEPS

Scripps Institution of Oceanography, La Jolla, California

V. A. KRASHENINNIKOV

Geological Institute, Academy of Sciences, Moscow

H. OKADA

Kagoshima University, Kagoshima, Japan

M. H. RUEF

State Department of Ecology, Olympia, Washington

We have constructed a kinematic model which outlines the deposition of the units of the western Pacific seafloor on growing crust. The average northward component of motion for the Pacific plate has been 4.4 cm yr⁻¹ from 100 to 30 m.y. BP and 2 cm yr⁻¹ from 30 m.y. BP to the present.

BENEATH the thin (20 to 100 m) unlithified layer of brown clay on the floor of the western Pacific abyss is a thicker (300 m) sequence of chalk, chert and clay, and the boundary between the two layers is broadly time transgressive, varying in age from Early Cretaceous in the west to Late Cainozoic in the east. Because there has been a tendency to attribute a precise synchronicity to pelagic facies, this evidence of widespread and apparently systematic diachronism is significant. Evidence of the time transgressive nature of pelagic deposits has been accumulating since the beginning of the deep sea drilling product¹⁻³, and during Glomar Challenger's Leg XX we extended observations of this phenomenon to the oldest known areas of the Pacific crust^{4,5}. We have now also a kinematic model of pelagic deposition for the time transgressive sedimentary sequences of the Pacific basin, which

may be applied to the stratigraphic succession on any oceanic plate.

A Mid-Latitude Model for Deep-sea Pelagic Deposition

There are at least three principal sources of deep-sea pelagic sediments—the remains of living organisms, terrigenous detritus supplied as wind blown dust or suspended matter, and pyroclastic material. The biogenic remains are composed predominantly of calcareous and siliceous tests which dissolve as they sink to and rest on the ocean floor. The solution of the tests is controlled by a number of variables which result in effective compensation depths below which no calcareous or siliceous remains are found^{6,7}. Thus the record of biogenic sedimentation on the ocean floor will depend on the depth relative to the appropriate compensation depth. By contrast the inorganic component may be found at all depths. Other variables such as plate motion and crustal subsidence are thus only important because they control the location of the plate relative to sources of supply, the pattern of oceanic circulation and depth relative to the compensation depths.

We propose that as new oceanic crust is formed in the rift valley of the Mid-Oceanic Ridge a blanket of light-coloured carbonate ooze is deposited on the new crust until the subsiding sea floor drops below the carbonate compensation depth. Subsequently a blanket of slowly accumulating abyssal clay covers the organic debris (Fig. 1).

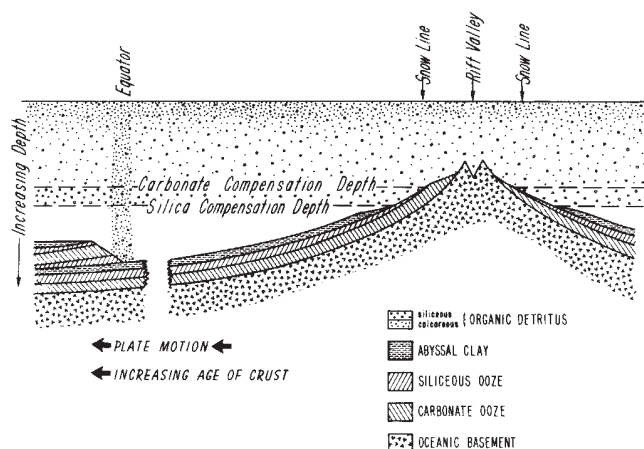


Fig. 1 Axially accreting model of oceanic sedimentation.

should have accumulated some 300 m of sediments consisting of 200 m of basal chalk and 100 m of overlying abyssal clay. Because the floor of the western Pacific is at least Early Cretaceous in age³⁻⁵ we may compare the drilling results with the simple model.

Sediment Sequences in the Western Pacific

The sea floor of the western Pacific is covered by 5 stratigraphic units: a wedge shaped layer of Quaternary to Late Tertiary silty clay primarily of volcanic origin, thinning away from the Asiatic volcanic-arc source; a ~100 m thick layer of Tertiary to Cretaceous abyssal zeolitic clay; a sequence (± 100 m) of Tertiary to Cretaceous cherts and chalks; an inferred earlier, thin abyssal clay a few tens of metres thick; and basal chalks, cherts, limestones and marls of Cretaceous age over 200 m thick (Fig. 2). Variations of this stratigraphy

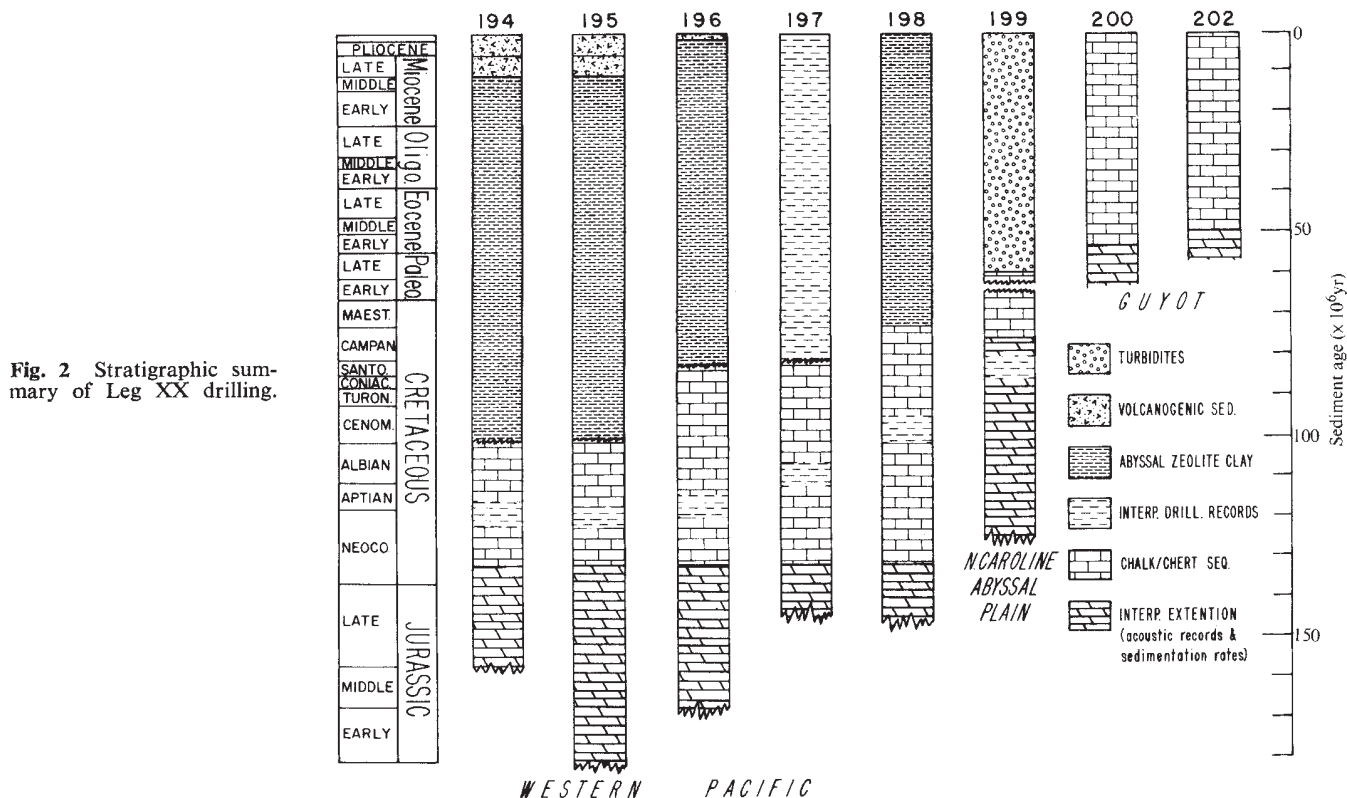


Fig. 2 Stratigraphic summary of Leg XX drilling.

The thickness and nature of the biogenic debris are determined by the depth difference between the compensation depth and the initial depth at the ridge crest, the rate of subsidence, and the type of biogenic sediments accumulating at any particular latitude. In general, the compensation depth lies about 1,000 m below the ridge crest, and new crust subsides about 50 m (m.y.)⁻¹, allowing 20 m.y. of biogenic deposition before the onset of abyssal clay sedimentation. Sedimentation rates for pelagic biogenic oozes are less than 10 m (m.y.)⁻¹. Thus the basal calcareous layers can normally be expected not to exceed 200 m in thickness. Sedimentation rates for abyssal clay are generally significantly less than 1 m (m.y.)⁻¹. Thus Early Cretaceous (120 m.y.) crust formed under ideal conditions

are seen in the Caroline Abyssal Plain, where Late Tertiary abyssal clays are intercalated with turbiditic calcareous oozes^{4,5}.

An Initial Comparison

The stratigraphic history may in part be explained as the normal sedimentary sequence expected to overlie a section of axially accreting mid-latitude oceanic crust. But the comparison of the mid-latitude model with the stratigraphic sequence drilled during Leg XX reveals serious discrepancies. Not only are the two chert/chalk sequences together nearly twice as

thick as predicted but the span of time from the first chalk to the last chalk is closer to 50 m.y. than the 20 m.y. given by the model. The simple model outlined applies specifically to the mid-latitudes and the mid-ocean, and may be modified by considering additional sources of sediments.

Equatorial Model

Anomalously high surface productivity may be expected to push the compensation levels ("chalk line") to much greater depths (Fig. 1) and may increase significantly rates of biogenic accumulation⁸. The most notable example of chalk-line depression occurs in a narrow zone between the northern hemisphere and southern hemisphere current systems,

diagenetic conversion to bedded chert. Because the borders of the equatorial belts of carbonate and siliceous ooze are known to have suffered extensive fluctuations in the Quaternary, we may assume that alternation of thin siliceous and thicker carbonate oozes is an overall characteristic of equatorial deposition.

In practice there seems to be a depth below which the chalk line may not be depressed. At present this depth lies near 5,000 m. Ocean depth is a function of crustal age; a depth of 5,000 m generally corresponds to crust more than 50 m.y. old. Thus crust older than 50 m.y. at the time of equatorial transit might be deprived of an equatorial deposit, or the transit may be represented by siliceous deposits or by a thin sequence of carbonates.

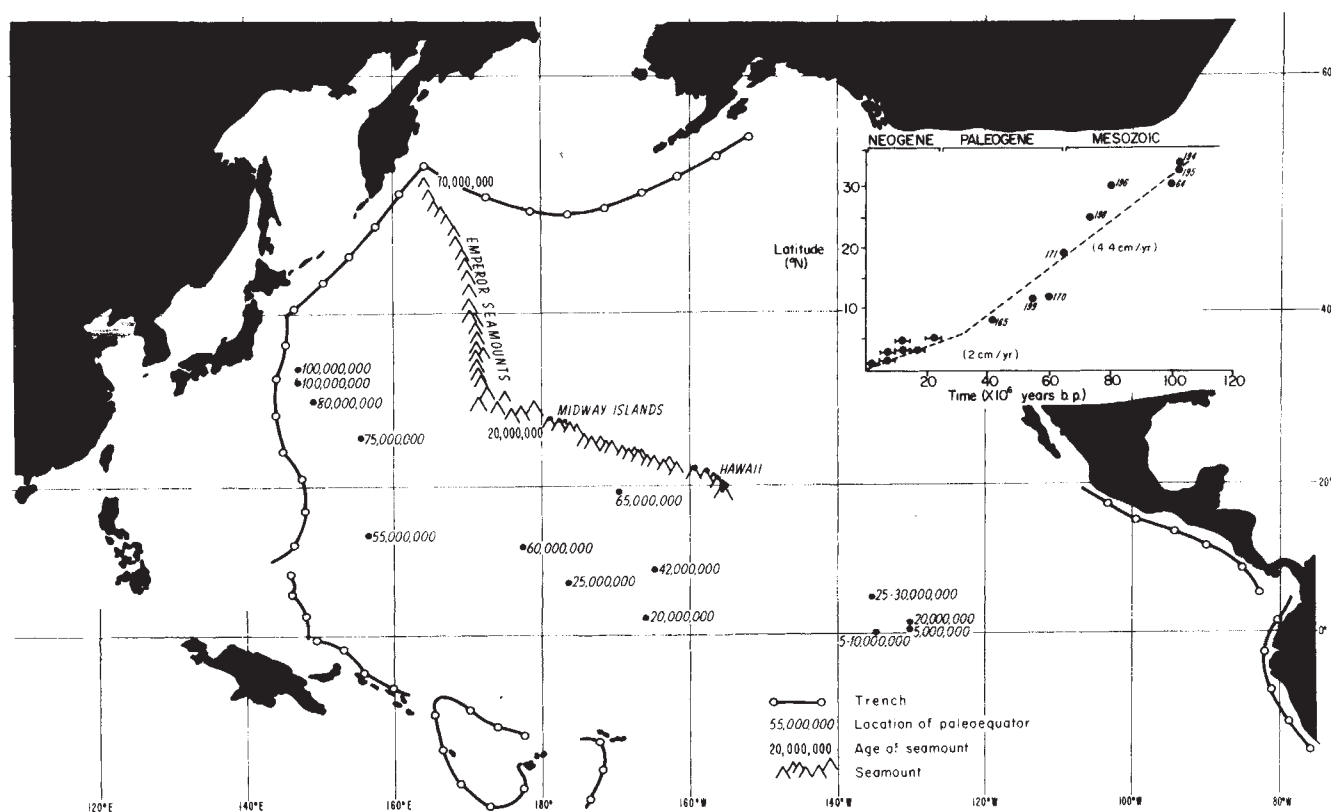


Fig. 3 Locations of palaeoequator in Pacific.

beneath the narrow zone of equatorial upwelling. Crust that forms under the equator may accumulate a considerable thickness of biogenic sediments. The specific thickness of sediments will depend on the rate and direction of crustal drift and the age of the crust at the time of equatorial passage^{9,10}. Crust drifting parallel to the equator may accumulate enormous thicknesses, particularly on young crust, but crust drifting north or south receives a thinner chalk whose thickness depends largely on the rate of drift.

A belt of siliceous ooze deposition lies between the equatorial zone of mainly carbonate deposition and the vast expanse of barren abyssal brown clay which floors the deep Pacific. The belt of siliceous oozes, at the margins of the Equatorial belt of high productivity, may be explained by the greater effective silica compensation depth, and allows us to predict the presence of siliceous units above and below ancient equatorial carbonates (Fig. 1). The resulting diachronous deposits of siliceous sediments would seem to be likely candidates for

The age of the chert/chalk to abyssal clay boundary may thus be used as an index of the equatorial transit (Fig. 3). When combined with other evidence for equatorial transits⁸⁻¹² and the suggestion for a major change in the Pacific plate trajectory at about 30 m.y. (ref. 13), average northward components of plate motion of 2 cm yr⁻¹ and 4.4 cm yr⁻¹ are obtained for the Late Tertiary and the Early Tertiary to Cretaceous, respectively. Similar evidence of northward plate motion has been inferred from studies of the remnant magnetism of cored basalts and seamounts^{9-12,14}.

Thus, the mid-latitude and equatorial mid-ocean pelagic models, when combined, compare favourably with the drilling results of the northern Pacific. The Cretaceous chert/chalk sequence that lies beneath the Tertiary and Late Cretaceous abyssal clay in the northwestern Pacific records the earlier Mesozoic passage of the ancient Pacific crust beneath the equator. The Lower Cretaceous chert/chalk sequence that lies at the bottom of the northern holes apparently represents

the initial basal chalk formed on new crust, and the abyssal clay that separates the 2 units records abyssal-basin sedimentation during the interval between the initial subsidence below chalk-line and the subsequent re-occurrence of chert/chalk deposition during the equatorial passage. The apparent success of the simple kinematic model in accounting for the stratigraphy of the western Pacific led us to examine more general implications of the model and to attempt a more analytical approach.

Analytical Model of Pacific Sedimentation

The following numerical values have been used in the calculation of the Pacific sedimentation models (Fig. 4): Crust formation at ridge crest at 3,000 m depth; compensation level at depth of 3,700 m; ridge crest subsides at rate of 35 m (m.y.)⁻¹; sediment accumulation rates of biogenic sediments at 10 m (m.y.)⁻¹; abyssal clay at 1 m (m.y.)⁻¹, and equatorial biogenic sediments at an additional 10 m (m.y.)⁻¹; zone of equatorial sedimentation 5° wide; trajectories of Pacific plate with a "westward" component approximately parallel to fracture zones, 259° and a northward component of 2 cm yr⁻¹ from 0 to 30 m.y., and 4 cm yr⁻¹ from 30 to 200 m.y.; and orientation of cross-sections at 79° intersecting 160° E at 0°, intersecting 160° E at 15° N, and intersecting 160° E at 30° N.

Basically the mathematics of the model is a simple book-keeping operation. We consider as an example a section perpendicular to the ridge crest. Whatever the relative motion of the equatorial zone, it may be described by its speed and width projected on the section. At each step in time, the sediments accumulated at each location are calculated from the appropriate sedimentation rates, and added to the sediment thickness already there. Isochrons may then be drawn through the thickness generated to produce a model geologic section. Several parallel sections based on the same parameters give a complete history of sedimentation and vertical traces may then be compared with a bore hole from any locality (Fig. 4).

A model has been calculated for a profile across the Pacific from the modern ridge crest off California to the Mesozoic crust off the Mariana Arc (Fig. 3) and may be compared with the gross lithic stratigraphy reported from Pacific deep sea drilling.

Summary of Pacific Stratigraphy from DSDP Data

The basic data are shown as vertical columns from drill holes projected to the lines of section (Fig. 4). Fig. 5 illustrates an interpolated summary of the distribution of the different major lithologies in the Pacific.

A comparison of the model (Fig. 4) with the observed stratigraphy (Fig. 5) and stratigraphic summary (Fig. 6) shows good agreement. The Pacific crust ages from east to west; an observation justifying a basic assumption of the model. The basal chert/chalk unit is time transgressive increasing in age from east to west. In the eastern Pacific the basal carbonate is about 50 to 200 m thick (or about 20 to 40 m.y. in extent) indicating a general agreement with the model. The basal chert/chalk is interpreted as that accumulated at the ridge prior to its submergence beneath the compensation depth. An upper chert/chalk unit which is only present in the western Pacific is also time transgressive, increasing in age from the equator northwards, and from east to west, and is interpreted as the biogenic oozes accumulated during the passage of the Pacific crust beneath the equator. The upper and the lower chert/chalk units merge in mid Pacific with the existence of a chevron pattern as predicted by the model. Two abyssal clay units are observed. The lower unit consists of a wedge-

shaped horizon, whose upper and lower boundaries are time transgressive. The base of the lower clay is the chert/chalk sequence accumulated at the ridge crest, and the top, the chert/chalk sequence deposited during the equatorial crossing. The upper clay unit is only found in the northern Pacific wedging out to the equator in the south, and the younger crust to the east.

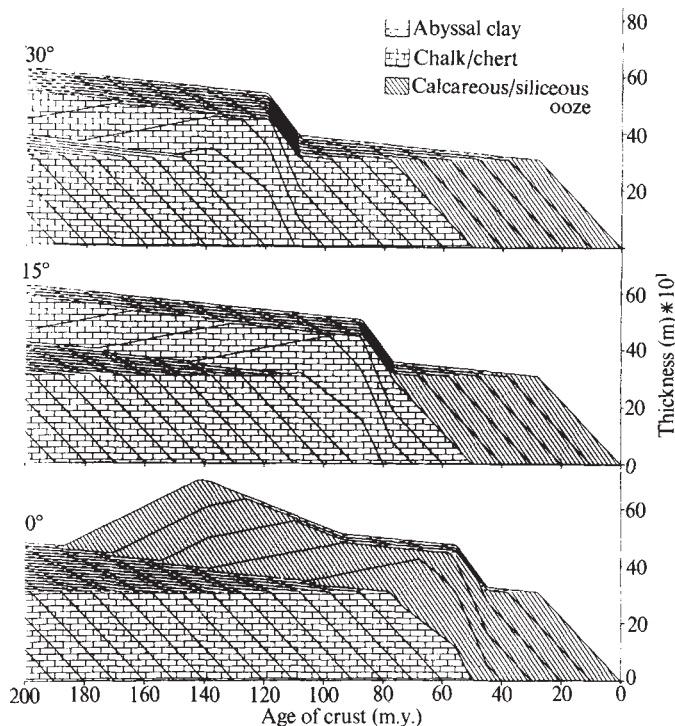


Fig. 4 Computed models of Pacific Ocean sedimentation history.

Other features observed in the data summary (Fig. 6) but not included in the model (Fig. 4) are that: Where the oceanic crust has passed marginal to a continental margin, positive area, or an extinct volcano we see the addition of turbiditic or volcanogenic sediments. Good examples are the wedge of turbidites along the west coast of North America¹⁷⁻²¹, the turbidites filling the Caroline Abyssal Plain^{4,5}, and the wedge of wind blown volcanogenic debris east of the Asiatic arcs^{4,5}. The presence of equatorial biogenic oozes along the whole length of the present equator, and the absence of this unit in any section south of the equator, and the onset of lithified chert/chalk sequences from calcareous and siliceous oozes at a crustal age of at least 50 m.y.

In general there is a close fit between the model and data summary both with respect to the distribution pattern, timing and thicknesses of the major lithologies. The fit is encouraging, and lends credence to the model as a useful first order approximation of the sedimentary history of the Pacific plate. Departures from the model should now illustrate smaller or more local events in the sedimentary history and add detail to the geologic history of the Pacific.

Acoustic Stratigraphy

The principal boundaries separating the lithologic units provide the strong contrasts in acoustic impedance necessary to create major reflectors of regional extent. Seismic reflexion profiling²² has defined five acoustic units in the Pacific; an upper transparent layer lacking strong reflectors; a unit

consisting of closely spaced strong reflectors known as the opaque layer; a lower transparent layer which is of smaller regional extent than the overlying layers; a lower opaque layer of limited extent; and basement, which, if smooth, is referred to as horizon B. The upper transparent layer corresponds to the abyssal clay in the west and central Pacific, turbidite sequences in the north and east, and unconsolidated biogenic oozes in drillings to the east and along the equator. The upper opaque layer corresponds to the thick chert/carbonate sequence of the western Pacific, the locally occurring lower transparent layer may correspond to the Cretaceous clay of deep sea drilling, and the lower opaque layer corre-

chert/carbonate sequence deposited at least in part under equatorial conditions. The area of the Pacific in which the upper opaque horizon is observed is that portion of the crust which has passed under the equator (Fig. 7). The limits of the upper opaque layer are the equator on the south, the subducting trenches on the west, and a line, marking the limit of crust created subsequent to the equatorial passage, on the northeast. The opaque layer is apparently thickest between 20° and 30° N, a fact which may be explained by a smaller northward component of drift of the Pacific crust in Early Cretaceous time, an effect similar to that recorded for Neogene time by the equatorial sediment bulge.

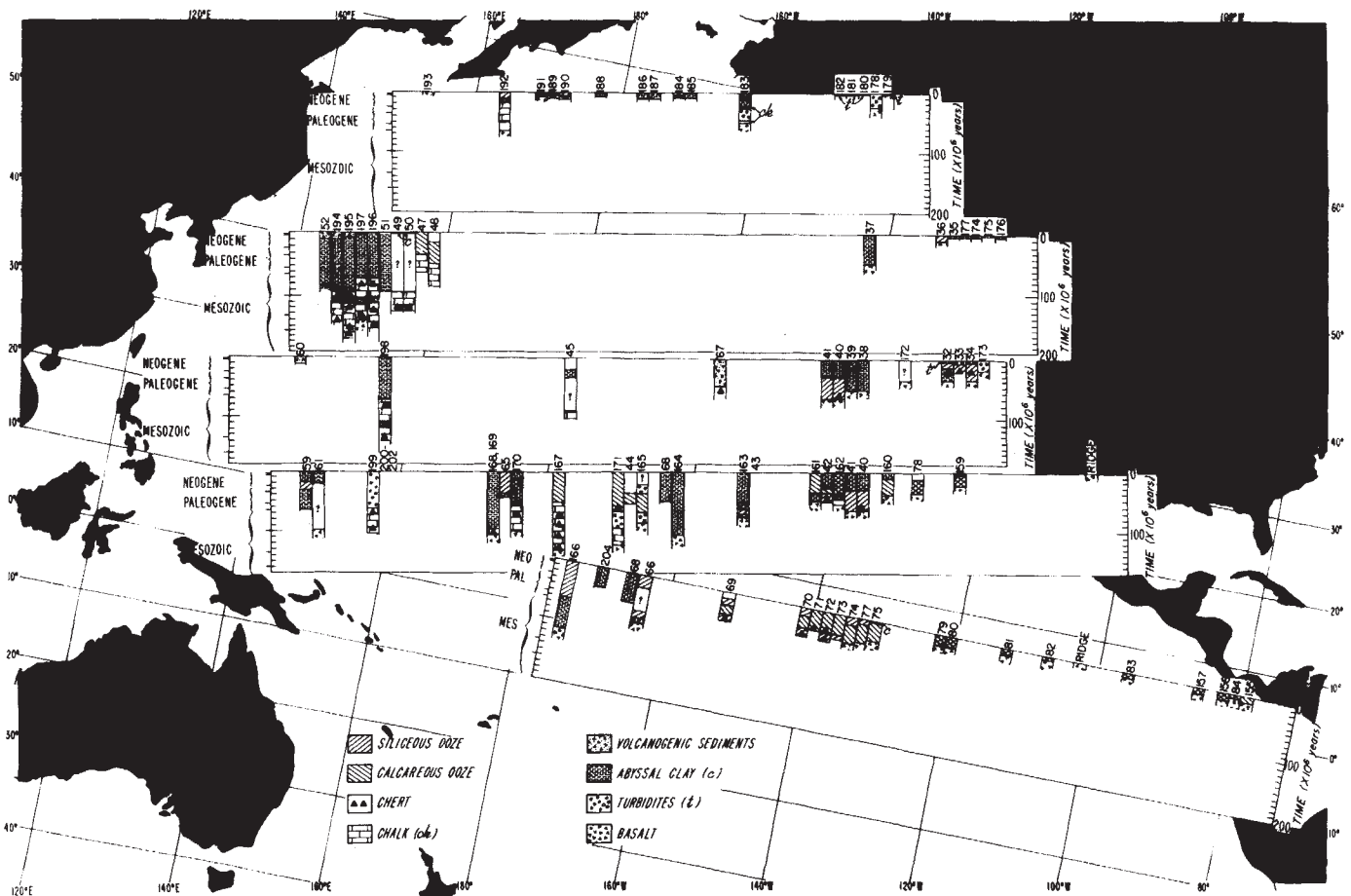


Fig. 5 Summary of drilling data from Deep Sea Drilling Project.

sponds to the basal chert/carbonate in areas which having crossed the equator have acquired additional carbonates higher in the section.

Previous interpretations of the acoustic records evidenced a strong preference for the synchronicity of acoustic boundaries (that is, lithic boundaries), suggesting Eocene or Late Maestrichtian ocean-wide, or even world-wide, horizons apparently resulting from synchronous global events.

The top of the opaque layer is, however, widely time transgressive. Thus the former assumption of synchronicity simply does not conform to the evidence from deep sea drilling and is in conflict with the kinematic model of lithologic stratigraphy. Similarly, the assumption that the opaque layer is a clastic wedge has been disproved by the drilling results.

The acoustic data, stripped of the synchronous time-stratigraphic interpretation, can be compared favourably to the kinematic model. The upper opaque layer evidently is a

The upper acoustic opaque layer cannot be mapped in the northernmost western Pacific for the practical reason that the lower transparent layer pinches out a few hundred kilometres northwest of the Shatsky Plateau. This pinchout suggests that the basal carbonates merge with the equatorial carbonates and cherts, because the period of time separating the original generation of this crust and the equatorial transit of that crust was too brief to be represented by a layer of abyssal clay thick enough to be detectable by seismic reflexion profiling. Because the rate of abyssal clay deposition is so slow that a layer only 20 or 10 m thick is equivalent to 10 to 20 m.y., the true pinchout could be a considerable distance northwest of that shown by reflexion records. Thus somewhat northwest of the lower transparent layer pinchout would seem to have been formed very near the equator in pre-Hauterivian time.

The acoustic stratigraphy thus seems adequately accounted for by the kinematic model.

Certain further predictions can be made: First, on crust of a given age the total thickness of pelagic deposits should be markedly less in the South Pacific, which has yet to receive an equatorial contribution, than in the North Pacific where equatorial deposition has already produced an additional layer of carbonates; second, south of the equator cherts should be much less common than to the north.

Thus the new kinematic stratigraphy of pelagic deposits which predicts a characteristically diachronous behaviour of

the acoustic upper opaque layer. The magnetic quiet zone has been explained as a period in the Cretaceous during which magnetic reversals did not occur²³. Although this explanation may be true in part, no data have yet been presented in the literature in clear support of this assumption. The kinematic model predicts that the magnetic quiet is the locus of crust formed under the equator. If there was indeed a period without reversals during the Cretaceous its effect would be to widen the apparent intersection and introduce other details.

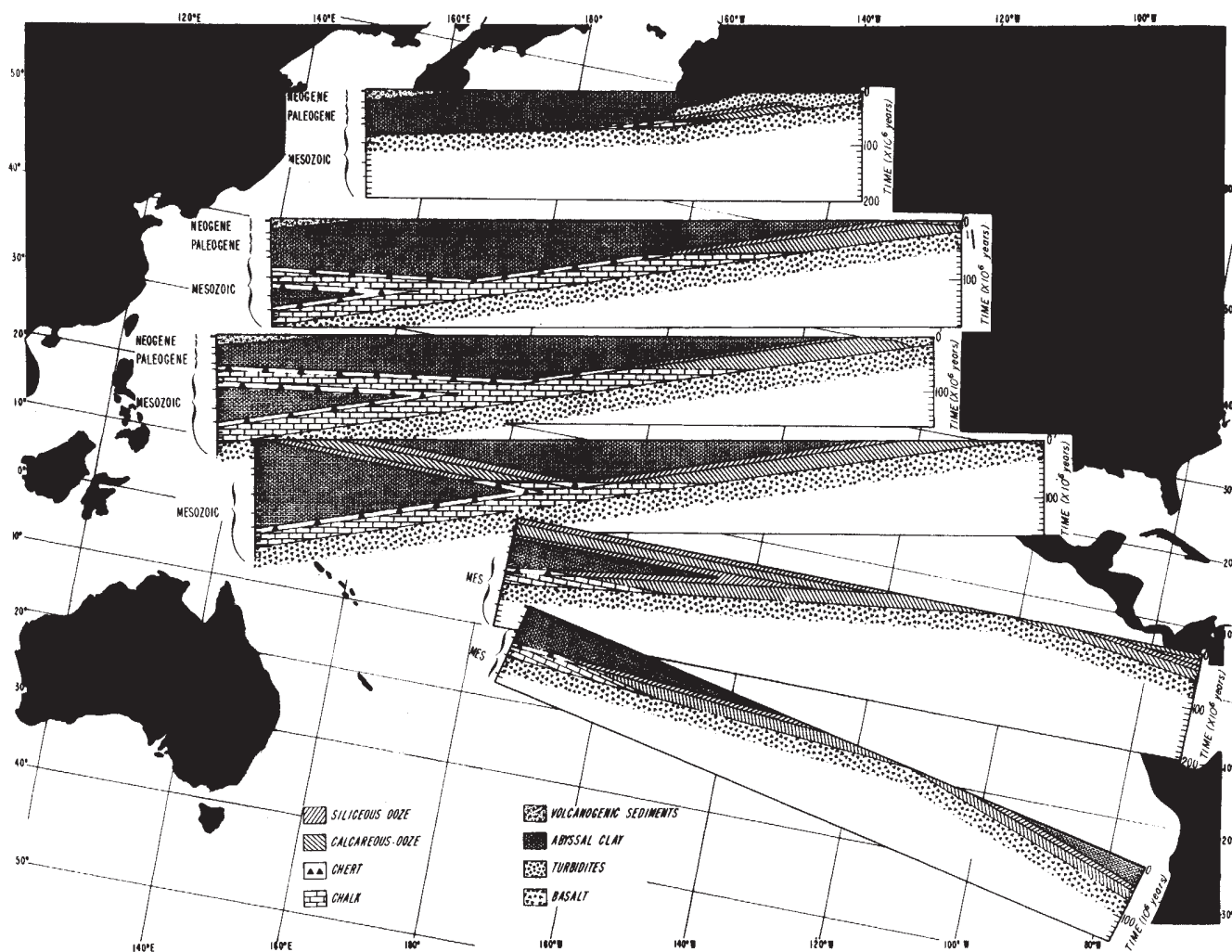


Fig. 6 Interpreted cross-sections showing sedimentary history of Pacific crust.

units can be applied to acoustic stratigraphy as well as to lithostratigraphy.

Magnetic Data

Stripe-like magnetic anomalies generated by east-west axial accretion are either absent or poorly developed in crust generated at the magnetic equator. A magnetically quiet zone has been mapped in the mid and high latitudes of the north central Pacific²³. The zone which extends through the eastern Pacific from the present intersection of the magnetic equator with the axial rift valley towards the northwest Pacific (Fig. 7), lies somewhat east of the easternmost sites which sampled equatorial carbonates, and just east of the limit of

The Pacific crust is divided into two regions, one formed before and a second after the equatorial crossing. The boundary between the two is the "magnetic quiet" zone (Fig. 7). Similarly, the restriction of thick opaque layers to the western Pacific results in the correlation of this region with that portion of the Pacific crust that has passed beneath the equator, and thus received a double layer of biogenic sediment.

Diachronous Detrital Deposits on the Margins of the Pacific Plate

Evidence of crustal motions may also be derived from diachronous volcanic deposits near plate margins. A detrital wedge of volcanic debris is found within 1,000 km of the

Asiatic arcs in the northwest Pacific (Fig. 6). The shape and extent of the wedge is a function of two counter balancing effects, the amount and extent of distribution of volcanic debris and the westward subduction of the Pacific plate. Assuming that the rates of both processes have been essentially constant during Late Tertiary time, equilibrium should result and the shape of the wedge would stay essentially constant. Near the distal edge of the wedge (sites 194, 195, 196), Late Tertiary sedimentation rates are approximately the same.

the northward migration of the Pacific crust beneath the palaeoequator.

While we are gratified by the apparent close agreement of data and model we wish to emphasize the severe limitations of both. The model presented here has ignored both the theoretical effect of reversals in plate motion on bed thickness and possible evidence of such reversals. We have considered only a few changes in direction and rate of crustal drift and ignored possible changes in sea level or compensation depth.

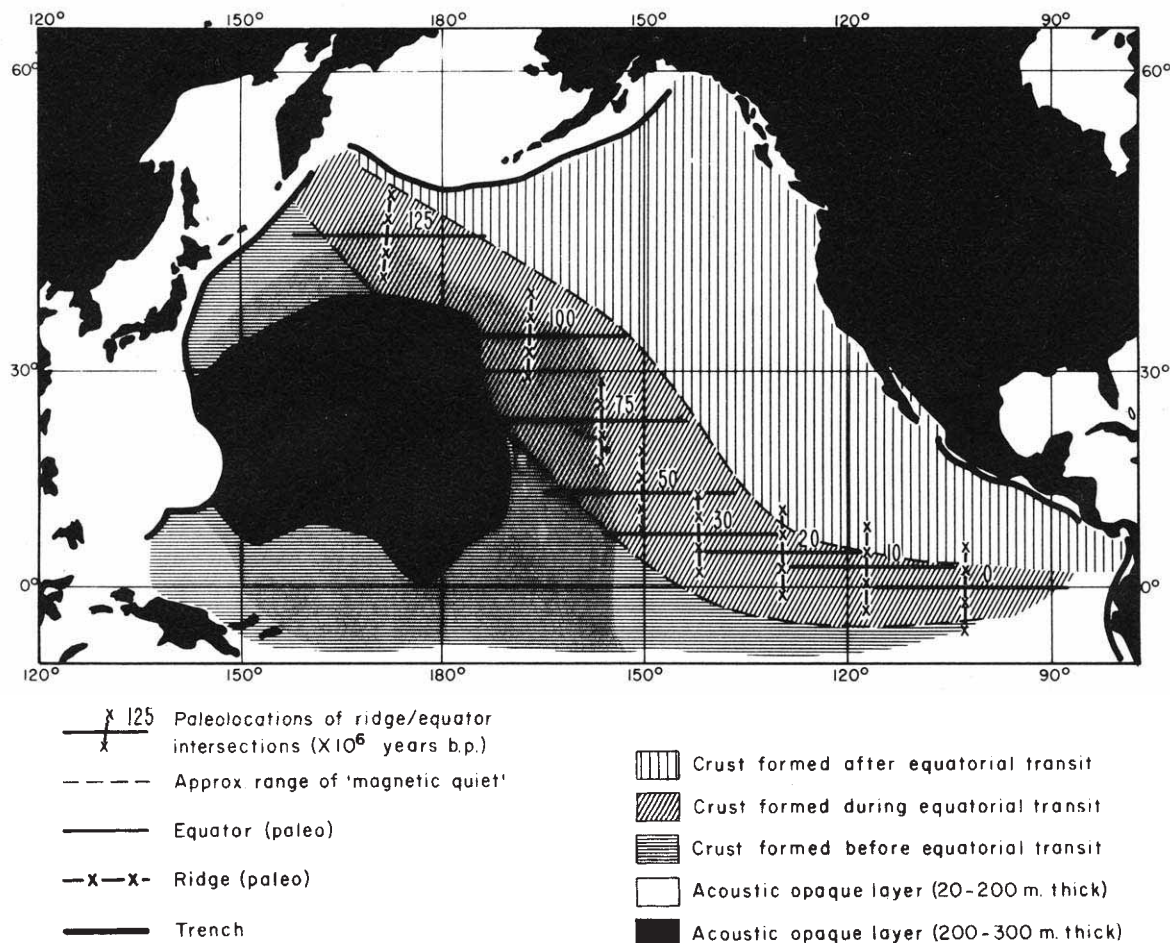


Fig. 7 Age structure of the Pacific crust.

Thus the difference in thickness of the wedge between sites may be ascribed purely to the older age of the initial deposition surface at the western sites. With the above assumptions a value of about 8 cm yr^{-1} is obtained for the westward component of drift of the crust, that is, the subduction rate into the marginal trenches. This rate is of the same magnitude as the axial accretion determined for the past few million years by the magnetic stripes at the crest of the Mid-Oceanic Ridge.

By contrast other marginal detrital deposits may be synchronous, that is, the cut off of turbidity currents to the relict Aleutian Abyssal Plain by the blockage of the Aleutian Trench²⁴, or the commencement of turbiditic deposition on the Caroline Abyssal Plain by the creation of a source area in the Caroline Islands.

Whereas detrital sediments deposited in the deep sea may by means of some catastrophic mechanism be broadly synchronous, the characteristic litho-stratigraphy of pelagic deposits is broadly and systematically diachronous. The history of the Pacific plate has apparently been governed by a few simple and systematic factors: the carbonate and silica compensation depth, the gradual sinking of oceanic crust and

In our opinion a relatively small number of additional holes drilled in the more ancient parts of the ocean would provide data for a greatly refined model. It would seem that even with only a few dozen better located, well sampled bore holes in the ancient Pacific plate we could, with the aid of a kinematic model, arrive at a rather detailed history of the Pacific since the Jurassic.

The Deep Sea Drilling Project is supported by the NSF and administered by the Scripps Institution of Oceanography. We thank W. A. Nierenberg, M. N. A. Peterson, N. T. Edgar and project staff for assistance.

Received July 14; revised August 29, 1972.

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DNA Replication Sites within Nuclei of Mammalian Cells

JOEL A. HUBERMAN, ALICE TSAI
& ROBERT A. DEICH

Departments of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

DNA replication can occur throughout the nucleus and is not restricted to the inner surface of the nuclear membrane.

THE involvement of a membrane site in DNA replication was first suggested by Jacob, Brenner and Cuzin¹ in their "replicon" model for replication of bacterial DNA, but the evidence accumulated since then is inconclusive. In prokaryotic cells, co-sedimentation of DNA replication points and cell membranes has been demonstrated¹⁻⁴, and the origin of replication and the membrane found to be associated⁵. A lipid-free replicating DNA-protein complex from *E. coli* has been isolated⁶ and it has been reported that only the origin, but not the growing points, of the *E. coli* chromosome is attached to membrane⁷.

In cell fractionation experiments with mammalian cells it was also found that replication points, detected by pulse-labelling with ³H-thymidine (TdR), are associated with the nuclear membrane (or some other large, light, hydrophobic cell structure)^{8-11,13}. Association between replication points and the nuclear membrane has also been detected by electron microscope autoradiography of thin sections through pulse-labelled, unsynchronized HeLa cells. The label associated with the membrane apparently moved into the nuclear interior after a 1 h chase¹³.

On the other hand, most electron microscope autoradio-

graphic experiments suggest that replication can take place throughout the nucleus. For instance, Comings and Kakefuda¹⁴ generally found grains located throughout the nucleus when unsynchronized human amnion cells were pulse-labelled for 5 min or more with ³H-TdR and then sectioned and autoradiographed. When, however, cells that were supposedly synchronized at the beginning of S phase were pulse-labelled for 5 or 10 min, grains were found predominantly over the nuclear membrane. Comings and Kakefuda concluded that initiation of replication takes place on the nuclear membrane, whereas the replication occurs anywhere within the nucleus.

Somewhat different results were obtained by Blondel¹⁵, using KB cells and pulse times of 2 min; Williams and Ockey¹⁶, using Chinese hamster cells and pulse times of 10 min or longer; and Erlandson and de Harven¹⁷, using HeLa cells and pulse times of 15 min. All three groups concluded, in agreement with Comings and Kakefuda¹⁴, that during a large part of the S phase grains are produced over the entire nucleus, but they differed from them in finding a peripheral pattern of grains more frequently at the end of the S phase than at the beginning. A cell fractionation experiment by Kay *et al.*¹⁸ also demonstrated association of late-replicating but not early-replicating DNA with the nuclear membrane.

Higher Resolution Autoradiography

Regardless of the time in S phase at which replication occurs close to the nuclear membrane, one important implication of

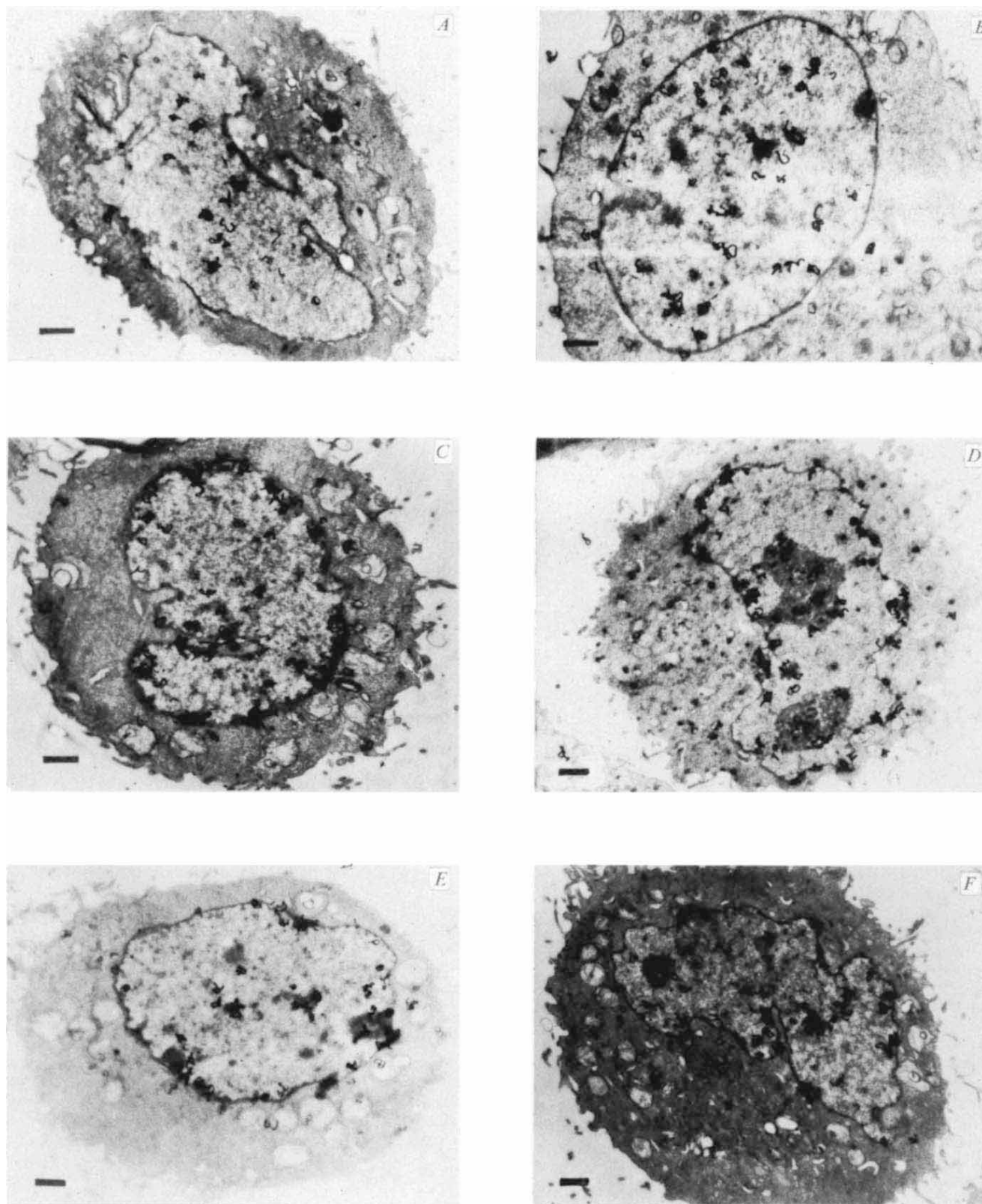


Fig. 1 Autoradiography of unsynchronized CHO cells exposed to ^3H -TdR for 0.5 min. *A* and *B*, grains distributed over entire nucleus. *C* and *D*, grains distributed around nuclear membrane. *E*, clustered grain distribution. *F*, mixed grain distribution. CHO cells were grown on Petri plates in Joklik-modified MEM (Grand Island Biological Company) supplemented with 7% foetal calf serum and non-essential amino-acids. Pulse-labelling was performed by first adding 5-fluorouridine deoxyriboside (FUDR; Hoffmann-LaRoche) to a final concentration of $1.6 \mu\text{g ml}^{-1}$ to inhibit further biosynthesis of dTTP. After 1 min, ^3H -TdR (51 Ci mmol^{-1} ; New England Nuclear) was added to $17 \mu\text{Ci ml}^{-1}$. After 30 s the plates were removed from 37°C , and the medium rapidly sucked off. The plates were then washed with ice-cold isotonic saline containing FUDR at $0.1 \mu\text{g ml}^{-1}$ (two changes). Cells were removed from the plates by trypsinization at room temperature in isotonic saline still containing FUDR. The cells were pelleted, then fixed with glutaraldehyde and OsO_4 , dehydrated, embedded in epoxy resin²⁹ and cut into gold-purple sections. The sections were mounted on grids and autoradiographed by the technique of Caro and Van Tubergen²⁰ except that the acetic acid stop bath was replaced with distilled water. Exposure time was 3.5 months. The bar in each figure represents $1 \mu\text{m}$.

most of these electron microscope autoradiography experiments is that, in some parts of S phase, at least, replication occurs throughout the nucleus. How can this implication be reconciled with the experiments indicating that all replicating DNA is associated with the nuclear membrane? It is possible that a considerable amount of DNA is synthesized during a pulse as short as 2 min. Huberman and Riggs¹⁹ have shown that the rate of DNA replication in Chinese hamster cells can be as much as $2.5 \mu\text{m min}^{-1}$, although most replication seems to occur at rates between 0.5 and $1.2 \mu\text{m min}^{-1}$. Thus as much as $5 \mu\text{m}$ of DNA could be synthesized during a 2 min pulse. If this DNA were synthesized at the nuclear membrane and then stretched out, it could reach the centre of a nucleus of diameter 5–8 μm and give rise to a false impression of the location of sites of DNA synthesis.

This effect could be ruled out by shorter pulses. We calculated that a pulse of 0.5 min would provide sufficient grains, after an exposure of several months, and the resolution (less than 1.25 μm of DNA synthesized) necessary to distinguish between replication solely at the nuclear membrane and replication elsewhere in the nucleus.

In most experiments, we used Chinese hamster (CHO) cells, which can be easily synchronized. The cells were pulse-labelled for 0.5 min with $^3\text{H-TdR}$, then washed, fixed, embedded, sectioned and stained. The sections were placed on grids and autoradiographed by standard techniques²⁰. After exposure times of several months, the emulsions were developed and the grids were examined by electron microscopy.

Sites of Replication

Cells from an unsynchronized culture, shown in Fig. 1, illustrate the variety of distribution of grains over the nucleus. The cells in Fig. 1A and B are labelled throughout the nucleus (general pattern), whereas the cells in Fig. 1C and D are labelled predominantly around the nuclear membrane (peripheral pattern). The grains in Fig. 1E are mostly clustered over regions of condensed chromatin, frequently near the membrane. Fig. 1F shows a cell with grains throughout the nucleus but with some concentration around the nuclear membrane.

Many grains are found in some cells more than 1.25 μm from the nuclear membrane (central grains), suggesting that replication is taking place at sites away from the membrane. To be certain of that conclusion, however, other possible explanations must be ruled out. The central grains cannot be due to background because few or no grains are found over the cytoplasm. They must be due to incorporation into DNA since no grains are found over G1 or G2 cells (see below), and the grains of isolated nuclei can be removed by DNAase. The central grains could also originate from DNA replication occurring on invaginations of the nuclear membrane either just above or just below the section plane. While this might explain some central grains, it certainly cannot explain most of them. Because the cells are sectioned with random orientations, we can get some idea of the frequency of invaginations which might bring nuclear membrane within 1.25 μm of the section plane from the frequency of invaginations in the nuclear periphery. Although some peripheral invaginations are evident in Fig. 1, they are not so frequent that most of the central area could be 1.25 μm or less from an invagination.

In some preliminary experiments we used HeLa cells, which have much more regular nuclei: central grains were also found over their nuclei (Fig. 2). Even serial sections up to 0.5 μm apart through a single cell all showed central grains. The change in pattern from general labelling in early S to peripheral labelling in late S suggests that general labelling is not an artefact of sectioning. Finally, the central grains cannot be accounted for by internal membranes within the nucleus. Such membranes have never been seen by other electron microscopists; apart from invaginations of the peripheral membrane we could see none in our sections.

To express quantitatively the proportion of cells showing

peripheral labelling, we have modified the method of Williams and Ockey¹⁶. We defined central grains as those further than 1.25 μm from the nearest nuclear membrane, and peripheral grains to be those closer than 1.25 μm to the nuclear membrane. The "central activity" of a cell section was calculated as the ratio of the fraction of central grains: the fraction of nuclear area which was central. Nucleolar areas and grains were excluded because the nucleolus has less DNA than the rest of the nucleus. A histogram showing the frequency of various central activities for an unsynchronized cell population is shown in Fig. 3 (a central activity of 1.0 implies an equal concentration of grains over central and peripheral areas).

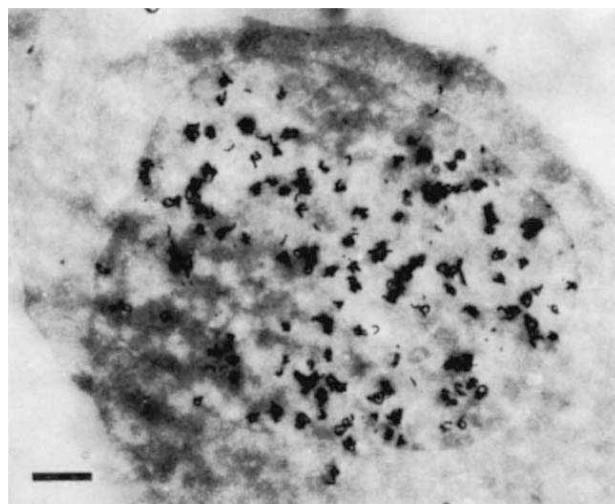


Fig. 2 Autoradiography of a HeLa cell exposed to $^3\text{H-TdR}$ for 1.5 min. The cells were grown in suspension culture in Joklik-modified MEM (Grand Island Biological Company) supplemented with 5% horse serum. Pulse-labelling was done by adding $^3\text{H-TdR}$ (20 Ci mmol^{-1} , New England Nuclear) to a concentration of 20 $\mu\text{Ci ml}^{-1}$. Exactly 1.5 min after addition of $^3\text{H-TdR}$, the pulse was terminated by addition of an equal volume of ice-cold isotonic saline containing 1% glutaraldehyde. Subsequent processing was as in Fig. 1. Exposure time was 9.5 months. The bar represents 1 μm .

Only 13% of the cells in Fig. 3 have central activities of less than 0.25, showing that DNA synthesis can go on at sites away from the nuclear membrane. The nuclear membrane is therefore not essential for DNA replication in Chinese hamster cells. But the fact that many cells do show a predominantly peripheral pattern remains to be explained.

Peripheral Replication in Late S Phase

Since previous autoradiographic studies^{14–17} had suggested that peripheral labelling occurs only at certain times during the S phase, we decided to try short pulse-labelling of cells synchronized to various times during the cell cycle. We chose 'Colcemid' reversal²¹ to synchronize the cells. Growing cells were treated with 'Colcemid' for a few hours and the cells blocked in mitosis collected by selective trypsinization. These cells (90–100% mitotic) were allowed to continue growing in the absence of 'Colcemid'. Within 2 h, 98.5% of the cells completed division, and by 18 h after release from mitosis, more than 70% of the cells had divided again. We preferred this synchronization method to methods involving starvation of nucleotides²¹, because cells recover excellently and normal DNA replication is not affected.

Table 1 and Fig. 4 show the distribution of grains within the nucleus as a function of time after release from mitosis. Peripheral labelling cannot be detected in the early stages of S but becomes predominant in later S.

Stable Location of DNA

If DNA is synthesized at the nuclear membrane (or other localized sites within the nucleus), then the DNA should move toward the membrane for replication and away from the membrane afterwards. The pattern of grains after a long pulse or pulse-chase should then be different from that after a simple short pulse. If, on the other hand, DNA replication can take place anywhere in the nucleus, then DNA movement is unnecessary and the grain pattern after pulse-chase labelling could be identical to that after simple pulse-labelling.

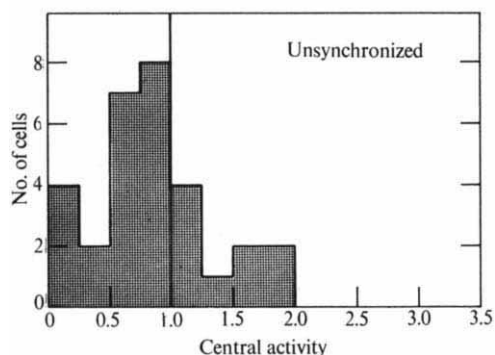


Fig. 3 Distribution of grains over unsynchronized CHO cells exposed to ^3H -TdR for 0.5 min. Prints at about $\times 15,000$ magnification were made of autoradiograms of individual randomly chosen cells similar to and including those in Fig. 1. A line was drawn within each nucleus which was at all points $1.25\ \mu\text{m}$ away from the nearest nuclear membrane. This line divided the nucleus into a central area and a peripheral area. Grains were counted over each area, and the size of each area was measured. In these determinations, grains lying over the nucleolus, and nucleolar areas, were ignored because the nucleolus has so much less DNA than the rest of the nucleus. Grains lying outside the nucleus were also ignored. "Central activity" was calculated as the ratio of the fraction of grains which were central to the fraction of area which was central. Thirty nuclei were measured for the histogram.

To test these possibilities, we first pulse-labelled synchronized cells for 0.5 min during the first half of the S phase (6 h after release from mitosis), and then chased with non-radioactive thymidine to late S phase (12 h after release from mitosis). Although the cells were examined in late S phase, they showed no higher proportion of peripheral labelling than expected for cells 6 h after release from mitosis (compare Figs. 4 and 5). In addition, unsynchronized cells pulse-labelled for 0.5 min

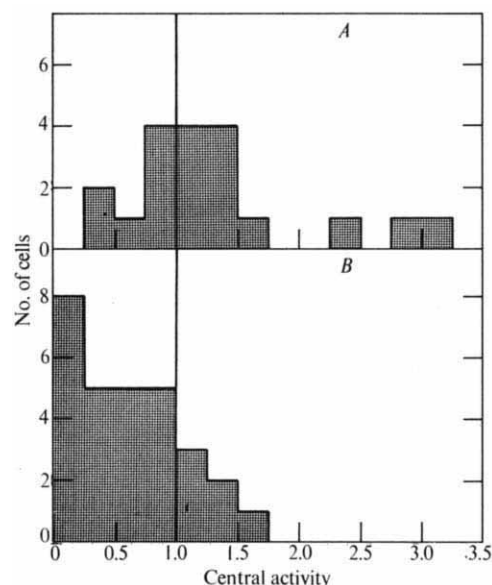


Fig. 4 Distributions of grains over synchronized CHO cells exposed to ^3H -TdR for 0.5 min. **A**, Early S phase (4–6 h after release from mitosis). **B**, Late S phase (10–12 h after release from mitosis). CHO cells were synchronized by the 'Colcemid' reversal method of Stubblefield. Pulse-labelling (performed at 2 h intervals after release from mitosis), preparation for electron microscopy, and autoradiography were performed as in Fig. 1. Exposure time was 2–4 months. Histograms were prepared as in Fig. 3. For **A**, eight nuclei pulse-labelled at 4 h and eleven nuclei labelled at 6 h after mitotic release were used. For **B**, thirteen nuclei labelled at 10 h and sixteen nuclei labelled at 12 h were used.

Hours after release from mitosis	2	4	6	8	10	12	14
Percentage of cells labelled *	26	83	93	89	81	62	48
Average number grains/cell section after 1 month exposure †	<1	6	16	16	24	17	—

*Cells were synchronized as in Fig. 4. At 2 h intervals after release from mitosis, ^3H -TdR ($20\ \text{Ci mmol}^{-1}$; New England Nuclear) was added to each plate ($17\ \mu\text{Ci ml}^{-1}$ final concentration). After 10 min the cells were washed twice with cold isotonic saline, collected by trypsinization at room temperature, allowed to swell in hypotonic medium ($2\ \text{mM MgCl}_2$, $1\ \text{mM EDTA}$, $10\ \text{mM KPO}_4$, $\text{pH } 7.7$) for 10 min, pelleted, fixed with methanol-acetic acid (3:1), spread onto subbed glass slides and allowed to dry. The slides were coated with autoradiographic stripping film ('Kodak AR-10') and exposed for 7 days. After development, the slides were stained with Giemsa stain, then mounted under 'Permunt'. At least 312 cells were scanned to determine the percent of cells labelled at each time point.

†The total number of grains over each nuclear section was determined for the autoradiographs used in Fig. 4. These figures were divided by the exposure time in months. Extra data for 2 h and 8 h after release from mitosis are included here.

show the same proportion of peripheral and general labelling as unsynchronized cells pulse-labelled for 10 min, or pulse-labelled for 0.5 min and then chased for 6.75 h (Fig. 5). In both of the chase experiments, the cells were more labelled after the chase than before, probably because incorporation of ^3H -TdR continues from the cells' internal pools.

Thus DNA has a stable location in the nucleus. Because some of the unsynchronized cells must have divided during the chase, our results suggest that DNA bound closely to the membrane in one generation is also bound to the membrane in the succeeding generation.

Because we cannot detect any difference in labelling pattern between a 0.5 min pulse and a 10 min pulse, our results can be compared directly with those of previous investigators^{15–17} who have used longer pulse times. Our results agree with most of the earlier results; a general-label pattern is obtained in early S phase and a peripheral one in late S phase^{15–17}, and chase experiments show that the nuclear position of DNA, once replicated, is stable^{15,16}.

The increased labelling during chase experiments, together with an apparently lower rate of synthesis in early S phase (Table 1), suggests an explanation for the autoradiographic results of O'Brien *et al.*¹³. In their pulse experiment, generally-labelled cells in early S phase may have been overlooked because they have few grains per nucleus (and a more disperse distribution at that); the increased labelling provided by the chase would have made the generally labelled cells sufficiently obvious to count.

Initiation of Replication

Our results also partially disagree with those of Comings and Kakefuda¹⁴. They reported that cells synchronized to the beginning of S phase showed solely peripheral labelling. Williams and Ockey^{16, 22} suggested that Comings and Kake-

fuda's results might be due to cell damage during the lengthy nucleotide starvation (24 h in the presence of excess thymidine and then 14 h in the presence of amethopterin) used to synchronize their cells. Indeed, the cytoplasm of the synchronized cells in Comings and Kakefuda's experiments contain many large vacuoles (indicating serious cell damage), whereas their unsynchronized cells have normal cytoplasm. Why cell damage should result in a peripheral labelling pattern is not clear. The unexpected pattern may be related to the finding¹² that polyoma virus infection causes mouse satellite DNA, which is normally late replicating²³⁻²⁵, to replicate before bulk DNA.

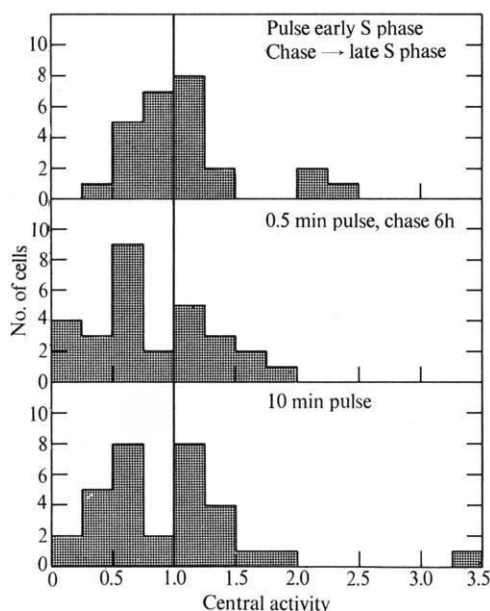


Fig. 5 Distribution of grains over CHO cells after pulse-chase or long pulse-labelling with ^3H -TdR. *A*, Synchronized cells (see Fig. 4) were pulse-labelled 6 h after release from mitosis as in Fig. 1. Then 30 s after addition of label, the medium was sucked off and the plates were washed, then replaced with medium containing $2.5 \mu\text{g ml}^{-1}$ of TdR. After 6 h the cells were collected by trypsinization and prepared for electron microscope autoradiography as in Fig. 1. Exposure time was 1.5–4 months. *B*, Unsynchronized cells were pulse-labelled as in Fig. 1 and chased for 6.75 h as in *A*. Exposure time was 3.25 month. *C*, Unsynchronized cells were pulse-labelled as in Fig. 1 except that the ^3H -TdR was left in contact with the cells for 10 min instead of 30 s. Exposure time was 1.5–6 months. In all cases, histograms were prepared as in Fig. 3. For *A*, twenty-four cells were used, whereas for *B*, twenty-nine cells were used, and for *C*, thirty-two cells were used.

Because Comings and Kakefuda's¹⁴ results must be discounted owing to cell damage, it seems that DNA synthesis can occur throughout the nucleus, and the nuclear membrane is not involved in initiation of replication. We have found, however, that in very early S phase (2 and 4 h after release from mitosis), the rate of replication per cell (measured as the average number of grains per nuclear section over labelled nuclei after a fixed exposure time) is much less than in later S phase (Table 1). This suggests that at the true beginning of S phase, the rate might be so low that we cannot detect labelling, let alone determine whether the pattern is peripheral or general.

Euchromatin and Heterochromatin

Heterochromatin has been shown to be replicated later than euchromatin and is condensed during interphase^{26,27}. Our sections and those of others¹⁴⁻¹⁷, show a preponderance of condensed chromatin attached to the inner nuclear membrane. Thus our results are consistent with the notion¹⁶ that euchromatin is replicated early in S phase and is distributed throughout

the nucleus, whereas heterochromatin is replicated in late S phase and is condensed along the inner nuclear membrane and in other discrete areas.

Reinterpretation of Cell Fractionation Experiments

Whereas many of the cell fractionation experiments on the intranuclear location of DNA synthesis^{8-11,13} have been interpreted in favour of attachment of replication sites to the nuclear membrane, this is not the only possible interpretation. Equally valid is the possibility that, after cell lysis, special structural properties of the growing points (extensive single strandedness, for instance) might result in their binding more protein, membrane, or other material than bulk DNA. Alternatively, the growing points may actually be attached inside the cell to some material that causes their separation from bulk DNA during lysate fractionation.

As experiments with mammalian^{8-11,13} and bacterial^{2,3,5} cells used similar techniques and gave the same results, it is possible that the growing point in bacteria may also not be attached to the membrane.

We thank Elaine and Robert Lenk for advice and assistance. This research was supported by grants from the National Science Foundation and the US National Institutes of Health. The electron microscopy was carried out in the Electron Microscope Facility of the Biology Department at MIT.

Note added in proof. Fakan *et al.*²⁸ have recently obtained results similar to ours in both autoradiographic and cell fractionation experiments with mouse cells.

Received May 4; revised December 15, 1972.

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LETTERS TO NATURE

PHYSICAL SCIENCES

2.8 cm Radio Emission from α Orionis, HBV 475 and MWC 349

WE have used the Max-Planck-Institut 100-m telescope to search for radio emission from stars that are believed to have ejected or to be ejecting matter. Eight known radio stars were on our observing list and all were detected. Three others, α Ori, HBV 475 and MWC 349, were also detected. Three others were possibly detected, but need further observations for confirmation. For 17 others we obtained no signal significantly greater than the r.m.s. noise. This communication concerns the three definite new detections.

The telescope was equipped with a cooled parametric amplifier at 10.68 GHz with an overall system noise temperature of about 120 K. The half-power-beam-width of the telescope is about 80 arc s and the aperture efficiency is about 40%. The pointing of the telescope is repeatable to a few arc s.

We used two observing methods. First, we made so-called on-off measurements in the beam switching mode. This results in doubling the signal of the source compared to conventional single-beam observations. The two beams were separated by 3 min 11 s and positioned symmetrically about the focus. The beams were pointed alternately to the star position. The total integration time for one "on" or one "off" was 2 min. At least four "on-offs" were made for each source. Second, if there seemed to be a positive signal in the beam-switching measurements, we scanned in two perpendicular directions through the position of the star. The length of a single scan was 10 arc min. After several scans the two horns were turned through 180 arc deg. In this way, the sign of the observed signal at the two beam positions in the scan was reversed. We consider the results obtained with the scan-method to be more significant than those obtained by the beam-switching technique; it is possible for random signals to simulate a positive detection in the beam switching, but it is highly unlikely that random signals will simulate the double beam response pattern in the scanning mode.

The data were obtained during the nights of October 28 and 31, 1972. The weather was stable and the nights clear. The r.m.s. noise after two minutes of integration corresponds to 4 mf.u. and is apparently due to system noise and not to confusion.

Table 1

Source	date	Method	$S_{10.68}$ (mf.u.)
α Ori *	72-10-29	On-off	9 ± 4
α Ori †	72-01-01	Scan	7 ± 1
HBV 475 *	72-10-28	On-off	12 ± 4
HBV 475 ‡	72-10-31	Scan	8 ± 1.5
MWC 349 ¶	72-10-28	Scan	460 ± 50
MWC 349 ¶	72-10-29	Scan	300 ± 40

* The error quoted is the r.m.s. deviation for a single on-off; twelve such measurements were made.

† The average of 240 scans; the error quoted is the r.m.s. deviation from the fitted double beam pattern (see Fig. 1a).

‡ The average of 120 scans; the error quoted is the r.m.s. deviation from the fitted double beam pattern (see Fig. 1b).

¶ The error quoted reflects the uncertainty in the calibration rather than being due to noise.

α Orionis: Weyman and Chapman¹ predicted detectable radio radiation from α Ori. Two searches were made in 1966 (refs. 2, 3) at frequencies close to ours. They gave some evidence of positive results on some occasions. Kellermann and Pauliny-Toth² obtained a positive result on one night, but eleven subsequent trials gave no detectable signal. The positive result obtained by Seaquist³ was apparently obtained during only one night. The signal to noise ratios of the detections never exceeded four. Further, the observations were all made with the on-off method only, with the inherent possibility of spurious signals. Thus, it remained uncertain whether the detections were real, or whether α Ori is a variable radio star.

The average of our azimuth scans through α Ori is shown in Fig. 1a. The flux derived is well below the values quoted by previous observers^{2,3}. We suggest that it represents the normal radio intensity of α Ori at this frequency, and that previous observers have detected outbursts in which the flux was temporarily higher. This suggestion is supported by the fact that we found no significant difference between the two observations made three days apart, whereas all other detections of radio stars have indicated that their fluxes are variable with time scales shorter than three days.

Our value for the flux from α Ori is less than that predicted by the model of Weyman and Chapman by a factor of 10. Hjellming and Wade⁴ obtained a negative result at 3.7 cm. If the flux at that wavelength is also a factor of 10 less than predicted, it would be just below their detection limit. Thus, it is possible that Weyman and Chapman's model correctly predicts the radio spectrum but is in error by a scale factor affecting the intensity.

If we assume a diameter for the source of 0.034 arc s, as quoted by Brown⁵ for the diameter of the star, it follows that the brightness temperature of the source must be about 10^5 K. If we assume, on the other hand, that the brightness tempera-

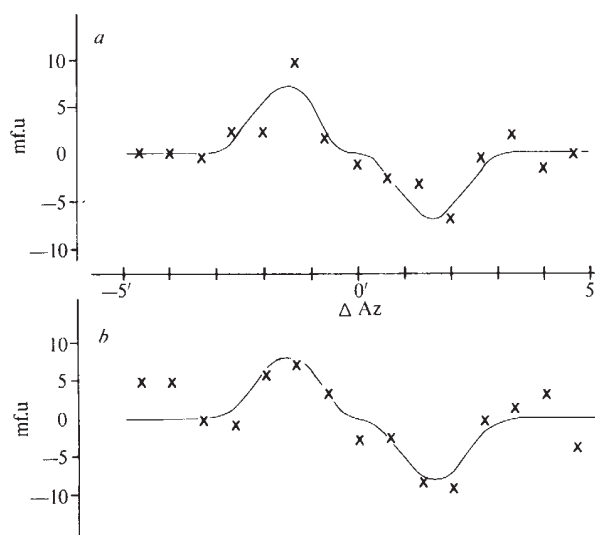


Fig. 1 Averaged azimuth scans for (a) α Orionis and (b) HBV 475. The fitted antenna pattern is shown as solid line, the observed points as crosses.

ture of the source is that of the photospheric temperature of the star, namely 3,500 K, we obtain a diameter of 0.18 arc s for the source, a value which is only about five times the diameter of the star. The real values of the size and brightness temperature of the source will certainly lie between these limits.

HBV 475: The variability of this star was discovered by Kohoutek⁶. Its spectrum is peculiar and contains emission lines⁷. Its light curve shows a slow but steady increase in brightness over the past 30 yr with the rate of increase being greater during the past five years. It seems likely that the increase in brightness is associated with an increase in the size of the star.

We detected radio emission on both of the two nights that observations were made (see Table 1). The average of our scans in azimuth is shown in Fig. 1b. We tentatively suggest that the radio emission originates in an extended atmosphere which also emits the optical emission lines.

MWC 349: A radio source within 1 arc s of the position of this peculiar Be star was reported by Braes, Habing and Schoenmaker^{8,9}. At 1,415 MHz they obtained a flux density value of 60 mf.u. We have the impression that the change in flux density from the night of October 28 to the night of October 29 is real, and that MWC 349 is a variable radio source, possibly of the Antares type.

We acknowledge the support of the technical divisions of the Max-Planck-Institut für Radioastronomie, and thank Dr I. Pauliny-Toth for supplying his stacking program and Dr L. F. Smith for reading the manuscript.

W. J. ALTENHOFF

Max-Planck-Institut für Radioastronomie,
53 Bonn, Argelanderstr. 3

H. J. WENDKER

Hamburger Sternwarte,
205 Hamburg 80,
Gojenbergsweg 112

Received November 27, 1972.

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5 GHz Observations of the Infrared Star MWC 349, and the H II Condensation W3(OH)

Braes, Habing and Schoenmaker¹ have detected radio emission from MWC 349, the flux density at 1,415 MHz being 0.060 ± 0.007 f.u. They also summarize the remarkable properties of this star, which is believed to be an early B-type star² with some 10 mag of obscuration and with a strong IR excess, which may be associated with a surrounding dense cloud of gas and dust^{3,4}.

We have observed the source with the 5-km radio telescope at Cambridge, and find it to have a flux density at 5 GHz of 0.22 ± 0.01 f.u. The source is slightly extended (Fig. 1), and by fitting a model to the two-dimensional visibility function may be approximated by a uniform circular brightness distribution with an angular diameter of 2.4 ± 0.2 arc s, although there is a weak extension in position angle 240° .

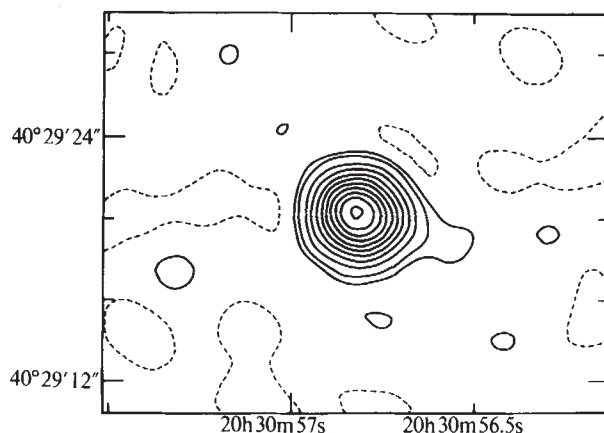


Fig. 1 Contour map of MWC 349 at 5 GHz. The contour interval is 0.0075 f.u. but the contours at $+\frac{1}{2}$ and $-\frac{1}{2}$ (dotted) of this interval are also shown.

The position of the centre is:

$$\left. \begin{array}{l} 20 \text{ h } 30 \text{ min } 56.84 \text{ s} \pm 0.01 \text{ s} \\ 40^\circ 29' 20''.38 \pm 0''.10 \end{array} \right\} 1950.0$$

The optical position of MWC 349 measured at Leiden is 0.9 arc s N of this position but new observations recently made by Yallop (Royal Greenwich Observatory) give a provisional position

$$\left. \begin{array}{l} 20 \text{ h } 30 \text{ min } 56.855 \text{ s} \pm 0.01 \text{ s} \\ 40^\circ 29' 20''.40 \pm 0''.1 \end{array} \right\} 1950.0$$

The agreement with this new position is very satisfactory. The angular size and flux densities of the source at 1.4 and 5 GHz are consistent with thermal emission from a region having an electron temperature (T_e) of 9,000 K and an emission measure of $2.7 \times 10^7 \text{ cm}^{-6} \text{ pc}$. The errors in the low frequency flux density and the angular size correspond to an uncertainty in T_e of $\pm 2,000$ K. The close similarity of this temperature to the value of 10,000 K found for many H II regions gives strong support for a thermal explanation for the radio emission from MWC 349.

Taking a distance of 2.1 kpc as suggested by Reddish⁵ on the basis of the possible association of MWC 349 with the Cygnus OB 2 association, the diameter of the radio source is 0.024 pc and the electron density $3.4 \times 10^4 \text{ cm}^{-3}$, corresponding to a mass of ionized gas of $9 \times 10^{-3} M_\odot$.

Because the source is optically thin at 5 GHz, we use the formula given by Rubin⁶ to obtain the total number of Lyman continuum photons emitted from such a region at a temperature of 9,000 K. This gives $L_c \sim 9 \times 10^{46} \text{ s}^{-1}$, suggesting that MWC 349 is of spectral type BO or earlier.

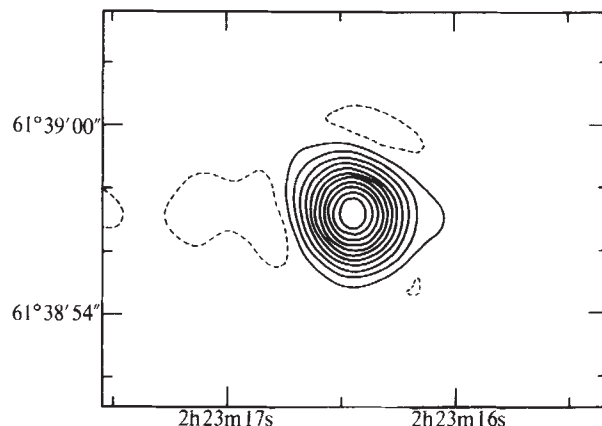


Fig. 2 Contour map of W3(OH) at 5 GHz. The contour interval is 0.040 f.u.

It is of interest to compare this source with the continuum emission from W3(OH), the compact component associated with OH emission in the H II region W3. This component has also recently been observed with the 5-km telescope (Fig. 2). The position of the centre is:

$$\left. \begin{array}{l} 02 \text{ h } 23 \text{ min } 16.44 \text{ s} \pm 0.01 \text{ s} \\ 61^\circ 38' 57''.18 \pm 0''.10 \end{array} \right\} 1950.0$$

The total flux density at 5 GHz is 0.62 ± 0.02 f.u. and nearly all of the emission originates in a compact elliptical component whose diameters are $(1.9 \pm 0.2 \text{ arc s})$ and $(2.3 \pm 0.2 \text{ arc s})$ with the major axis in position angle of about 60° .

Earlier observations of W3(OH) by Wynn-Williams⁷ with the 1-mile telescope showed that the source had dimensions of $<4.5 \text{ arc s} \times <5.2 \text{ arc s}$. He derived a model which fitted these observations and accounted for the spectrum. Assuming a distance of 3 kpc and an electron temperature of 10^4 K he concluded that the source was of diameter 0.024 pc and contained a total mass of ionized hydrogen of $0.042 M_\odot$.

In the light of the new data these figures must be slightly revised; the compact component has dimensions of $0.027 \times 0.033 \text{ pc}$. Because the compact component is optically thick at 5 GHz (ref. 7) the observations may be used to derive an electron temperature of $10,000 \pm 1,000 \text{ K}$. Using the spectrum of Wynn-Williams *et al.*⁸ we obtain a central emission measure of $\sim 4.7 \times 10^8 \text{ pc cm}^{-6}$, and a mass of ionized gas of $0.068 M_\odot$. Using the 86 GHz point shown on the spectrum in ref. 8 we obtain $L_c \sim 2.5 \times 10^{48} \text{ s}^{-1}$, indicating that there is probably a central exciting star of type O7 or earlier.

Cooper, Davies and Booth⁹ have measured the relative positions of 16 components of the 1,665 MHz emission from W3(OH). Although the positions are not absolute the mean position determined by Raimond and Eliasson¹⁰ for the 1,665 MHz emission lies within a few arc s of the centre of the continuum source. We suggest that the OH components determined by Cooper *et al.* may be located around the periphery of the continuum source as shown in Fig. 3. This distribution strongly supports the model of stimulated OH emission proposed by Cook^{11,12} who concluded that the emission will occur only where there is a long path-length in the line of sight in a medium of low temperature and small velocity dispersion; at the same time the region must lie close to the boundary of the H II region. The observed absence of

OH sources within the disk of the H II region, and their confinement within a narrow strip round the edge agrees well with this model.

We thank Dr David Allen (RGO) for discussions and Dr Andrew Murray (RGO) for providing us with the new position for MWC 349 prior to publication. One of us (C. S. H.) is indebted to the Science Research Council for a maintenance award.

J. E. BALDWIN
C. STELLA HARRIS
M. RYLE

*Mullard Radio Astronomy Observatory,
Cavendish Laboratory,
Cambridge, England*

Received December 4, 1972.

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Solar Electrical Discharges

Hemenway *et al.*¹ state, "We conclude that most of these submicron particles have come from the Sun, although this hypothesis poses formidable problems". These problems do not arise if the white streaks which can clearly be seen in the perimeter of sunspots are electrical discharges, because the temperature at points between or remote from these discharges at their level is low enough for particles to form and radiation pressure would cause some of them to be ejected. The presence of atmospheric particles generally leads to the formation of electrical charges².

E. W. CREW

*26 St David's Drive,
Broxbourne,
Hertfordshire*

Received October 5, 1972.

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Response of a General Circulation Model of the Atmosphere to Removal of the Arctic Ice-cap

OVER the past few years, numerical models have been developed for investigating the general circulation of the atmosphere and studying its long term behaviour. (See, for example, Smagorinsky *et al.*¹, Kasahara and Washington².) These models are firmly based on the equations of fluid motion and thermodynamics and simulate in mathematical terms the chief physical processes which are thought to be of importance in determining large scale atmospheric motions over long periods of time (a month or more). The advent of very high speed computers has made numerical experiments with these models reasonably easy.

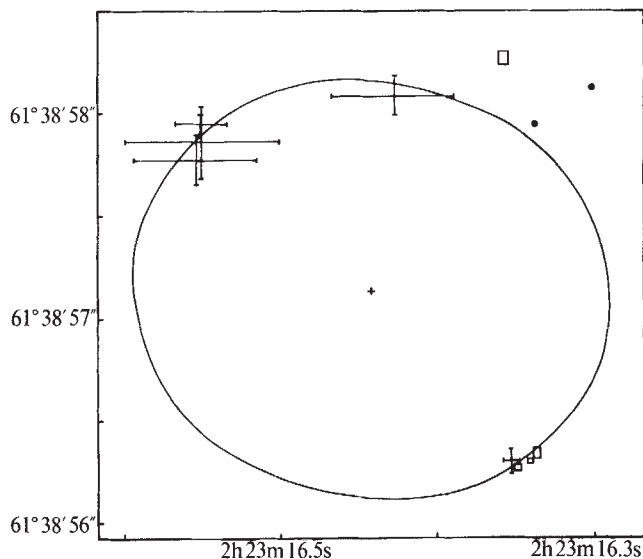


Fig. 3 Map showing the elliptical model of the W3(OH) continuum source fitted to the relative positions of the OH components⁹. The errors in the relative positions of the OH sources are indicated by error bars; the errors in the radii of the ellipse are $\pm 0.1 \text{ arc s}$ in any position angle.

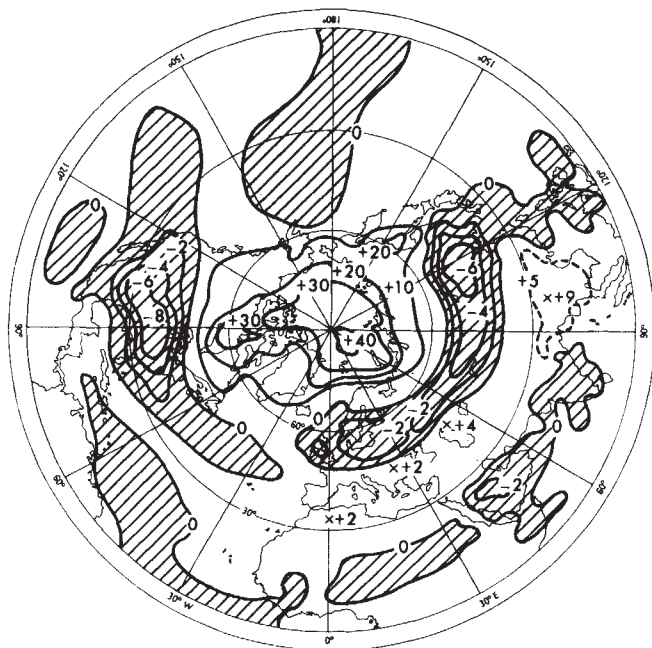


Fig. 1 Temperature differences, in °C near the model surface, between the computation with an ice-free arctic and the computation with ice at the mean climatological position. Hatched areas indicate regions of cooling in the ice-free experiment.

One model has been described in detail by Corby *et al.*³. The mode of computation essentially consists of integration forward in time from an initial state for a considerable number of model days using physical parameters appropriate to a given season and obtaining the mean seasonal state by averaging the later computed states; in the experiments described here the model was integrated forward in time for 80 days under winter conditions and the mean seasonal state obtained as the average of the last 40 days.

It is possible to vary the model to determine its reaction to substantial changes in the assumed physical properties. In one of these variants the region of winter arctic ice, as defined by the mean climatological position, was replaced by open ocean maintained at freezing temperature; what had been a surface with a temperature determined by radiational balance became a surface of constant temperature. The results of computations with ice and with open ocean differ quite considerably. The most marked difference is, of course, the great warming of the lower layers of the troposphere over the arctic basin, where, in the ice-free arctic experiment, there was an oceanic reserve of heat to balance radiative losses; this was to be expected. It might also have been expected that in mid-latitudes temperatures in the lower atmosphere would be raised. In fact, there is a lowering of mid-latitude continental temperatures near to the surface but with little change over the sea where the mean sea surface temperature had been left unchanged. This result is illustrated in Fig. 1, which shows the temperature difference between the two computed seasonal means at the level in the model most representative of the surface. The changes are considerable. The mid-latitude cooling may be compared with the temperature anomaly of -5°C in the very severe January of 1963; a temperature anomaly of -2°C would indicate a very cold winter month.

Other results from these experiments show a distinct southward displacement and weakening of the prevailing mid-latitude westerlies when there was no polar ice-cap, and this is similar to the result obtained from another ice-free arctic general circulation experiment conducted with the Mintz-Arakawa model⁴. What seems to happen is that the general decrease of temperature gradient between equator and pole reduces the strength of westerly flow in mid-latitudes

and, there, the circulations become more blocked. Consequently regions which at present benefit climatically from the westerlies might be cooler in an ice-free arctic regime.

I do not claim that these results necessarily indicate what might happen in the real atmosphere, because the model itself has several shortcomings, and one should not dwell on the results in particular areas because they are almost certainly not quantitatively correct. The sign of temperature changes in mid-latitudes is perhaps rather unexpected, and the results illustrate that qualitative arguments may be deceptive and unreliable in considering a physical system as complicated as the atmospheric circulation which includes so many non-linear interactions and feed-back mechanisms.

Considerable refinement and elaboration of numerical models will be required before their answers to questions of the kind raised in this letter can be accepted with any confidence. Nevertheless the potential superiority of the numerical approach for the investigation of problems of climatic modification is beyond doubt.

R. L. NEWSON

*Meteorological Office,
Bracknell, Berkshire*

Received October 23, 1972.

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Deep-sea Sediment Source Areas: Implications of Variable Rates of Movement between California and the Pacific Plate

RECENT work has indicated acceleration of motion on the San Andreas Fault¹, but previous studies have not delineated variable rates of motion with time between the Pacific and American plates. Offsets on the San Andreas and associated fault systems yield a mean rate of motion for a given period of time and deal with motion within the area of a wide plate boundary; therefore, these do not show total relative motion or variations in velocity between the two plates². Magnetic anomalies can be used to measure the total relative motion and variations in velocity between the two plates. But magnetic anomalies at the mouth of the Gulf of California represent only the past few million years of movement which has been going on for at least 25 m.y. (ref. 3). Here I describe a method by which the amount and rates of motion between California and the Pacific plate with time can be determined.

The Delgada submarine fan lies off northern California, south of the Mendocino Fracture Zone, and is at least Oligocene in age⁴. The fan lies in a unique position on the eastern edge of the Pacific plate directly adjacent to the varied lithology of source areas on the American plate to the east. If the fan has passed source areas of distinctive lithologies on the American plate, then its stratigraphy may indicate the amount and rate of movement between the two plates. This area is particularly well located for such a study because there is no land mass (source area) between the fan and the margin of the Pacific plate and because the Mendocino Fracture Zone inhibits the introduction of sediment into the fan area from the north⁴. Therefore changes in sediment composition may indicate the position of the fan relative to these distinctive source area rocks on the American plate.

Semi-quantitative X-ray diffraction studies of the less than 2 μm fraction⁵ from sites 32, 33 and 34, Leg V, of the DSDP, reveal significant changes in the percentages of the various clay minerals with time. The changes in percentages of kaolinite + chlorite/illite/montmorillonite occur at approximately 4 m.y., 5 m.y., 9 m.y. and 15 m.y. (Table 1).

Changes in the detrital coarse fraction mineralogy, amphibole and pyroxene assemblages and changes in degrees of stress and strain indicated by stretching and undulatory extinction show significant changes during these same time periods (manuscript in preparation).

Table 1 Computer Analysis of Maximum Variations in Percentages of Clay Minerals from Leg V, Sites 32, 33 and 34, DSDP

Absolute age (m.y.)	Chlorite and/or kaolinite	Illite	Montmorillonite
0			
2	48%	42%	7.5%
4			
6		30%	26%
8	32%	52%	16%
10			
12	Clay minerals occur as a trace component of the less than 2 μm fraction		
14			
16	14%	42%	46%

The Delgada fan is assumed to be one basin of deposition and to have been built by turbidity current and hemipelagic deposition from continental source areas. A simple model of evolution of the fan can be constructed so that the changes in mineralogy and style of deformation of mineral grains are consistent with the changing source areas (Fig. 1). Important source areas in this model, schematically represented in Fig. 1,

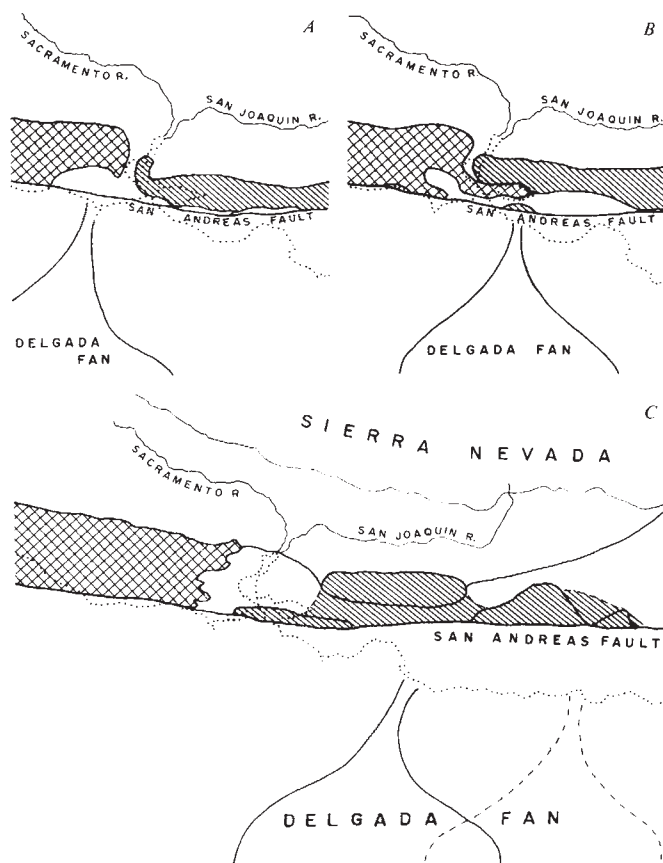


Fig. 1 Palaeogeography of central California⁶⁻⁸. Cross-lined represents northern Coast Ranges, lined the Diablo Range and eastern Santa Cruz Mountains, the dotted line is the present coast line. The Sierra Nevada Mountains would be outside the north margin of parts A and B. A, The fan at 4 m.y.; B, at 5.5 m.y.; C, at 9 m.y. (—) and 15 m.y. ago (---).

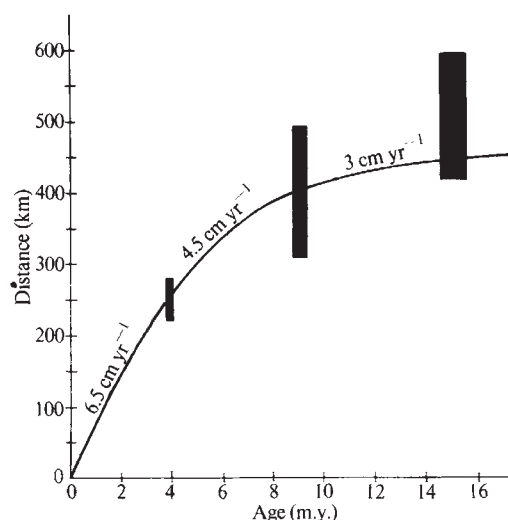


Fig. 2 Increasing rate of motion between California and the Pacific Plate. The ordinate is distance along the San Andreas Fault from the head of Delgada Canyon; the abscissa is absolute age of the fan sediment. The curve represents my preferred interpretation. Shaded areas are possible range of values: 5.5 to 7.0 cm yr^{-1} from Holocene to 4 m.y., 3.4 to 5.5 cm yr^{-1} from 4 to 9 m.y. and 2.8 to 4.0 cm yr^{-1} from 9 to 15 m.y. ago.

include: Northern Coast Ranges from Holocene to 4 m.y.; north-central Sierra Nevada from 4 m.y. to 5 m.y.; eastern Santa Cruz Mountains–northern Diablo Range from 5 m.y. to 9 m.y.; and southern Diablo Range–southern Sierra Nevada from 9 m.y. to 15 m.y. ago.

The greatest interpretative problem is: how far the fan travelled into a proposed source area before the influence of that source area dominated the fan sediment. The transition from one lithology to another in fan sediment is variable, spanning as little as 200,000 yr at the 4 m.y. change to as much as 1.5 m.y. at the 9 m.y. lithology change. Arbitrarily using the centre point of these time intervals and the palaeogeographic lithologic boundaries allows a preferred interpretation of rates of motion with time between California and the Pacific plate (Fig. 2). In all cases distances to varying lithologies shown in Fig. 1 are measured from the head of Delgada Canyon.

The rate of motion of 5.5 to 7.0 cm yr^{-1} for the past 4 m.y. yielded by this method agrees with the rate calculated from marine magnetic anomalies³ and adds validity to the choice of space-time boundaries. Reasonable variations in the placement of the palaeogeographic boundaries and possible errors in time boundaries would give ranges of rates of motion shown in Fig. 2. Even if the preferred interpretation is not correct, the data strongly suggest an increase in the rate of motion between California and the Pacific plate from about 16 m.y. to the present.

I thank T. Atwater, D. Bukry, R. Coe, R. Garrison, G. Griggs, J. Sumner, and S. Wright (Leg V, DSDP) for help.

JAMES R. HEIN

Earth Sciences Board,
University of California,
Santa Cruz, California 95060

Received September 21, 1972.

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Remarkable New Silicate Structure

Pabst, Gross and Alfors¹ described a new hydrated calcium silicate, named rosenhahnite after its discoverer. Lath-like single crystals up to 5 mm long were obtained and the morphology and physical characteristics measured. The composition of the crystals was found to be $\text{Ca}_3\text{Si}_3\text{O}_8\text{H}_2\text{O}$ with two units in a triclinic unit cell. Thermal dehydration data showed that the molecule of water is given off very slowly at temperatures between 400 and 500° C and a single crystal of rosenhahnite transforms into an almost perfect single crystal of wollastonite, CaSiO_3 , which is known to contain infinite Si_3O_8 chains. No visible cracking takes place and interfacial angles change little if at all, but the originally clear crystals become opaque and porcellaneous. From density measurements there is apparently about 5% of voids in the converted crystals, but these fissures are not large enough at the surface to admit toluene molecules. The perfect topotactic relation of the two lattices was established (apart from minor details) by the same investigators from partially transformed crystals, which gave almost equally sharp X-ray reflexions from both parts of the specimen.

The transformation to wollastonite is, of course, irreversible (except possibly under high pressure, hydrothermal conditions¹) and the crystal structure which could explain this transformation posed an intriguing problem as well as promising to throw more light on related materials of

importance to cement chemistry. Crystals of rosenhahnite were kindly supplied by Professor Pabst to this laboratory for a structure determination. This has just been completed and the remarkable behaviour of the mineral is, in fact, explained by a remarkable structure.

The structure determination was carried out using over 3,000 independent intensity measurements obtained from a 4-circle diffractometer with $\text{MoK}\alpha$ radiation. These intensities were checked against six layers photographed on a Weissenberg camera. A combination of Patterson synthesis and phase determination by direct methods was required to establish the structure. The tetrahedral co-ordination of O round Si was assumed in the early interpretations, but no assumptions were made about the relative arrangements of the tetrahedra or of the Ca atoms. The structure has refined to give a conventional discrepancy index (*R*-value) of under 4%, using isotropic temperature factors.

The model clearly shows distinct linear (but kinked) groups of three SiO_4 tetrahedra, the two outer silicon-atoms each sharing a common oxygen with the central one. There is no sign of any separate water molecule. One of the three terminal oxygens at each end of the group has a significantly longer bond to Si than the other two and has significant bonding to only one Ca, as against two or three for the others. The only significant peaks in the difference Fourier map occur at just under 1 Å from these oxygen atoms. It is clear that the difference Fourier peaks are the hydrogen atoms, and that OH is attached to each of the terminal silicon atoms. Fig. 1 shows a projection of the structure on the plane perpendicular to the *a* axis.

The groups of three SiO_4 tetrahedra must be $\text{Si}_3\text{O}_8(\text{OH})_2$ and the dehydration process consist in the polymerization of these groups into infinite Si_3O_8 chains, each join producing a molecule of water, and local rearrangements giving channels through which these molecules can diffuse.

The determination of the detailed mechanism of the transformation requires an accurate structure of wollastonite. A new set of data has been collected in this laboratory from triclinic wollastonite and the structure refinement is in progress. The results will be reported elsewhere. At present all that can be said is that, from the orientation relationships already established¹, the length of the $\text{Si}_3\text{O}_8(\text{OH})_2$ groups in rosenhahnite is in the general direction of the *b*-axis of the wollastonite into which it transforms, and this is the direction of the Si_3O_8 chains.

While the structure and polymerization process have no direct relation to cement chemistry, there is one aspect which may be indirectly relevant. It is now generally agreed that the strength of set cement derives to a considerable extent from physical bonding, but there is some evidence that a significant proportion of chemical bonding may also be present. It has been suggested² that chemical bonds might occur if foils or ribbons of the calcium silicate hydrate formed in setting cement came into contact so that the two lattices were aligned. Over a small area "welding" of the two foils might occur. No attempt to calculate the probability of this juxtaposition occurring was made, but it might be expected to be small. However, if a similar process merely required —SiOH groups on the two surfaces to come together, to form a bond by the elimination of water, the probability would obviously be much greater, and greater still if silicate ions were available to help bridge across gaps. Such speculations at least show the possibilities opened up by this exciting new structure.

J. W. JEFFERY
P. F. LINDLEY

Department of Crystallography,
Birkbeck College,
University of London,
Malet Street,
London WC1E 7HX

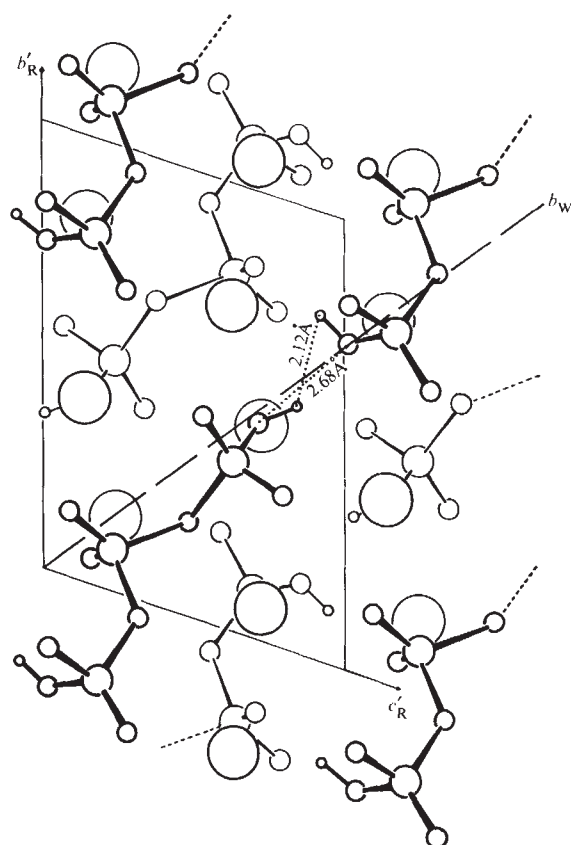


Fig. 1 The structure of rosenhahnite projected on to the plane normal to the *a* axis. b_R and c_R are the projected rosenhahnite axes. The circles, in order of decreasing size, represent Ca, Si, O and H. The direction of b_W , the *b* axis of the wollastonite into which the crystal transforms on dehydration, is shown by the long dashes. A short dashed line indicates the direction of the bond to the remainder of an incomplete $\text{Si}_3\text{O}_8(\text{OH})_2$ group. The dotted lines connect the oxygens and hydrogens of a pair of hydroxyls which clearly form hydrogen bonds and almost certainly condense together, liberating a molecule of water, in the polymerization process. All the $\text{Si}_3\text{O}_8(\text{OH})_2$ groups run nearly parallel to the projection plane.

Received September 13; revised October 9, 1972.

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Stability of Di-imide

DI-IMIDE, $\text{HN}=\text{NH}$, as the prototype azo compound and an isoelectronic analogue of C_2H_4 and O_2 , is of great interest to the chemist. It is, however, generally considered to be so unstable as to have only a transient existence at ordinary temperatures¹⁻³. Generated *in situ* in aqueous solution, it has been widely used in recent years as a reducing agent³, but has never been isolated or detected in these systems. It has been detected as a transient in the gas phase in several laboratories. Foner and Hudson⁴ produced di-imide by an electric discharge through flowing hydrazine vapour, detected it by mass spectrometry, and showed that it could be trapped at -196°C and regenerated in the gas phase on warming. Trombetti⁵, in these laboratories, was able to measure the infrared and vacuum-ultraviolet absorption spectrum of the vapour in a similar flow system, and these two studies seemed to indicate a lifetime of at least a second or two. A brief account by Mock⁶ suggests that the lifetime might be much longer; he observed that when di-imide vapour together with ammonia and hydrazine was admitted to the inlet manifold of a mass spectrometer, a signal at $m/e=30$ decayed with a half-life of several minutes and remained detectable for as long as 20 min. Uncertainty and lack of reproducibility of these observations led Mock to conclude only tentatively that di-imide might be as long lived as these data suggest.

We have carried out a detailed study of the chemistry of di-imide, and have found the lifetime in the vapour phase to be surprisingly long, substantially in agreement with Mock's observations. Di-imide was generated by a microwave discharge in hydrazine vapour in a flow system. Hydrazine was trapped out at -78°C , while ammonia and di-imide condensed in a second trap at -196°C , the di-imide forming a ring of bright yellow solid just above the liquid nitrogen surface. The second trap was then isolated, and di-imide, together with about a ten-fold excess of ammonia, could be vaporized by rapid warming and its reactions observed in a static system. The vapour shows a weak "azo" absorption in the near ultra-

violet (320–420 nm) which, unlike the spectra of most azo compounds, has a pronounced banded structure. This absorption provided a convenient means of measuring the concentration; Fig. 1 shows a typical decay of di-imide vapour at 30°C measured in this way in a cylindrical spectrophotometer cell 2 cm in diameter and 10 cm long. The decay in this instance was first-order with a half life of 2.4 min. The decomposition of the vapour is reproducible but complex, both in its kinetics and stoichiometry. It depends on pressure and surface, and can follow either first or second-order kinetics depending on conditions. Products of the decomposition are N_2 , H_2 , N_2H_4 and probably NH_3 .

Solid di-imide was found to be very stable, with no appreciable decomposition observed up to the melting point of the solid $\text{NH}_3\text{-N}_2\text{H}_2$ mixture at -65°C . Di-imide also appeared to persist for many minutes at least in the liquid mixture with ammonia at temperatures between -65° and -30°C .

A detailed account of these studies will be published elsewhere.

C. WILLIS*
R. A. BACK

Division of Chemistry,
National Research Council of Canada,
100 Sussex Drive, Ottawa

Received October 2, 1972.

*Present address: Chemistry Department, University of the West Indies, Kingston, Jamaica.

¹ Sidgwick, N. V. S., *The Chemical Elements and their Compounds*, 711 (Oxford University Press, London, 1950).

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BIOLOGICAL SCIENCES

Ecology of the Ras Muhammad Crack in Sinai

CRACKS running through raised Pleistocene coral reefs form a common environment around the tropical Indian and Pacific Oceans. The cracks can be reached only when they have an opening to the surface of the dry raised reef, and therefore the biota found in the open cracks is a mixture of light-avoiding subterranean species and of shadow-loving marine species. Near the southern tip of the Sinai Peninsula, Cape Ras Muhammad (Fig. 1), an open crack of about 40 m length and 0.20–1.5 m width and about 150 m inland, was discovered in October 1971 (Fig. 2). It is possible, with difficulty, to descend into the crack and dive to depths of over 14 m.

Contact with the sea is proved by the fact that the water level in the crack fluctuates with the tides, and the salinity of the water is about equal to that of the open sea: 23.60 g l.⁻¹ against 23.52 in October 1971, and 22.60 g l.⁻¹ at the surface and 22.80 g l.⁻¹ at 11 m depth against 23.60 g l.⁻¹ in the neighbouring lagoon.

The steep and shaded walls of the crack are covered with many algae, among them *Valonia*, *Codium*, *Botryocladia* and calcareous red algae, and sponges, monascidians and terebellid and serpulid polychaets. The fauna is dominated by two species of shrimp. According to Holthuis (personal communication), one is a new species of *Periclimenes*, a transparent pinkish, fairly active species, the other is a new genus of the family

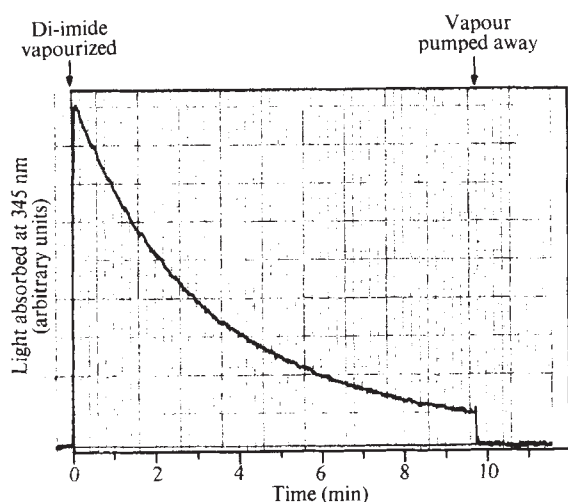


Fig. 1 Absorption of light at 345 nm by di-imide vapour, showing the disappearance with time at 30°C . Initial absorption was about 5%, and initial N_2H_2 pressure probably around 10 torr (an accurate extinction coefficient has not yet been established).

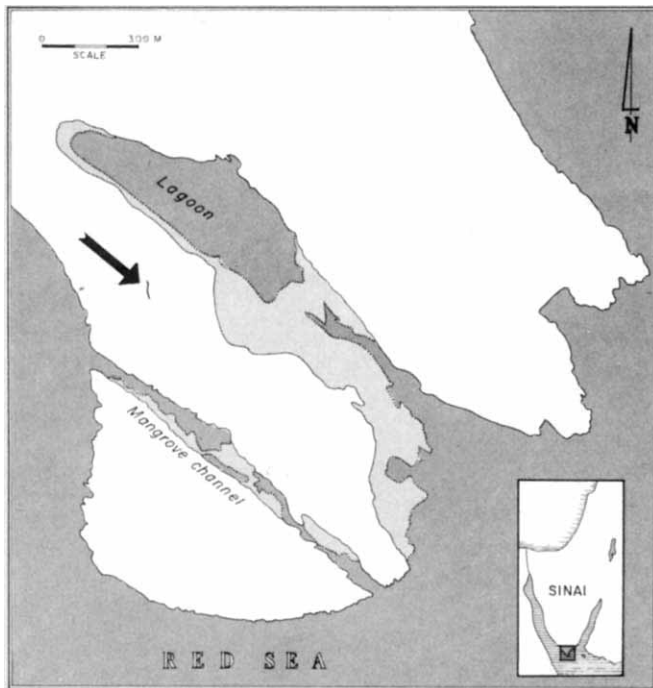


Fig. 1 Cape Ras Muhammad. Arrow points to the site of the "crack".

Hippolytidae, a bright red, blind species. A gobiid was the only fish observed. Swimming above the bottom there are many sphaeromatid isopods. There are large numbers of a *Cardita*-like bivalve, of a very high-shelled keyhole limpet, of an *Acanthopleura*-like chiton and even a few *Spondylus* were found. The sandy sediment accumulated at the shallow

northern end of the crack harbours a rich meiofauna including harpacticoids, the ostracod *Bairdia* and tanaidaceans.

Most of the inhabitants of the crack must have come through the subterranean connexion with the open sea. Blown-in leaves of turtle grass and crusts of a *Dictyosphaerium*-like alga could supply a second means by which marine animals, or rather their attached eggs, might reach the crack.

Holthuis¹ described this widespread type of environment as "tropical land-locked saltwater pools". Some species typical to this environment are the atyid shrimp *Antecaridina laevis* (Edmondson), reported from Devil's Crack (Dahlak Archipelago), Island of Europa and Fiji Islands, or the hippolytid shrimp *Ligur uvae* (Borradaile), reported from Aldabra, Halmahera and Fiji Islands¹. It was thus not quite unexpected that the same species of the new hippolytid genus of Ras Muhammad recently turned up in collections sent to L. B. Holthuis (personal communication) from Funafuti (Micronesia) and Hawaii. The only reef crack which has been more thoroughly investigated is Devil's Crack on the Island of Entedebir (Dahlak Archipelago, Southern Red Sea)¹⁻³.

The environment of the coral reef cracks is of worldwide occurrence in the tropics, a new subterranean watery medium in which a change to life in darkness and variable salinity takes place. This recent environment and these distributional patterns may shed some light on the genesis of certain subterranean shrimps, such as the genus *Typhlocaris*, which is represented today by three isolated species around the Mediterranean⁴.

We thank A. Amir and A. Levi for their technical assistance and Dr L. B. Holthuis of the Rijksmuseum in Leiden, Holland, for information on the shrimp.

F. D. POR
M. TSURNAMAL

H. Steinitz Marine Biology Laboratory,
Elat,
Department of Zoology,
Hebrew University of Jerusalem

¹ Holthuis, L. B., *Zool. Mededel.*, **38**, 16, 261 (1963).

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Fig. 2 Southward view of Ras Muhammad Crack.

Chromosomal Localization of Human Haemoglobin Structural Genes: Techniques Queried

Price, Conover and Hirschhorn¹ have reported the *in situ* hybridization of haemoglobin mRNA to diploid chromosomes. We think such a conclusion to be impossible because the specific activity of their mRNA was 6×10^{-7} disintegrations per mRNA molecule during the two week exposure of their autoradiographs. As a genome has been reported to contain less than five haemoglobin cistrons², even if hybridization and autoradiographic efficiencies were 100%, then at most only 6×10^{-6} silver grains would have been produced over the diploid cell's haemoglobin loci.

They reported up to six grains over the B chromosomes after a two week exposure and it is difficult to believe that these grains were due to specific mRNA hybrids. From their data in Fig. 1, the specific activity of their mRNA was 5 c.p.m. μg^{-1} . Wimber and Steffensen³ used 5S RNA at 4×10^6 d.p.m. μg^{-1} and after *in situ* hybridization to *Drosophila* polytene chromosomes, they detected only 20 grains per month over the 5S band. Such a band contains $200 (\text{redundancy}) \times 1,000 (\text{polyteny}) \times 2 (\text{diploidy}) = 400,000$ cistrons. One can easily see why the mRNA of Price *et al.*¹ was not radioactive enough to detect mRNA cistrons in a diploid chromosome even if the haemoglobin locus is a million-fold redundant.

We also suggest that instead of selecting eight cells out of 1,000 for analysis, one should show the frequency distribution of grains over as many of the cells as is practical. The unlabelled cells are as valid an experimental sample as the presumably "labelled" ones and excluding them from the analysis is a biased sampling procedure. A bacterial ^3H -RNA control and a poly A competition are also necessary for proper analysis of these results.

Department of Pathology,
Sloan Kettering Institute,
New York, NY 10021

W. PRENSKY

Department of Biological Chemistry,
Harvard Medical School,
Boston, Massachusetts 02115

G. HOLMQUIST

Received July 10, 1972.

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Do Bacteria Have a Nuclear Membrane?

THE idea of Stanier and van Niel¹ that bacteria (and possibly blue-green algae) are "prokaryotic" organisms, lacking a nuclear membrane, appears to have been accepted almost without argument. All other cells possess such a membrane, and are "eukaryotic", according to this theory.

The inner layer of the bacterial cell envelopes, however, which is commonly regarded as comparable with the semipermeable membrane of other cells, carries the attachments of the flagellum and the nuclear system. In "eukaryotic" protista it is with the nuclear membrane that such structures are associated².

Bacterial murein³ has been described⁴ in this journal as a layer between the cell wall proper and the cytoplasmic membrane; "the meat in the sandwich", which most appropriately consists of a lipoprotein complex. Although this may, and probably does, differ in chemical detail from the composition of classical cell membranes, and it is not alike in all bacteria, above the membrane, which is performing two of the most important known functions of the primitive nuclear membrane, there is a structure that, in some cases, at least chemically, suggests a membrane more than a cell wall, and could fairly be regarded as having been evolved from a previously existing, semipermeable membrane.

I suggest on the basis of general morphological relationships⁵ that the structure which now represents the cell membrane of bacteria may have originated in the nuclear membrane of an ancestral form; with the corollary that the murein layer could be derived from the cell membrane, the functions of which had been taken over by the nuclear membrane. The differences which may now exist between the composition of these organs, and that of their hypothetical forerunners, are no greater than those of many comparable examples, in the wider field and longer scale of evolution (for example, the retinal rods from cilia).

There is no reason to presume that bacteria, because of their small size, are in any way primitive⁶. They are, indeed, highly specialized organisms, making use of their small size, and consequent low bulk-surface ratio and rapid metabolism. They are almost certainly derived from other, more primitive, flagellate protista, and the origins of their organs must be sought in the general structural pool.

This hypothesis gives rise to further questions; the most obvious of these is: what service is such a transformation,

whereby the cell membrane reinforces the wall, while the nuclear membrane takes over its function? The best available answer refers back to the advantages of a small size and a large surface area, as the latter can be maintained, against the relatively strong forces of surface tension, in an already exceptionally small cell, only by great rigidity in the cell envelopes.

It may be that the supposed resemblance between bacteria and blue-green algae in having no nuclear membrane will prove to be as invalid as the previous theory; that they were alike in having no nucleus at all. They are alike mainly in their small size, and in the convergent adaptations that this produces. Positive groupings, based on negative criteria, are seldom durable in biology, and separate creation, even of organs, must have some evolutionary background.

K. A. BISSET

Department of Bacteriology,
The Medical School,
University of Birmingham,
Birmingham, B15 2TJ

Received June 16; final revision October 12, 1972.

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Can Mitochondrial Complementation be Used as a Tool in Breeding Hybrid Cereals?

SEVERAL papers¹⁻⁶ have been published in which it is claimed that mitochondrial heterosis and mitochondrial complementation are related to the yield potential of F_1 hybrid cereal cultivars. Mitochondrial heterosis is measured by the superior oxidative and phosphorylating efficiency of mitochondria isolated from heterotic F_1 hybrid cultivars when compared with those isolated from the corresponding parental cultivars. Although these parameters can be measured only after a small quantity of F_1 hybrid seed has been produced they may prove useful to plant breeders as a preliminary appraisal of the yield potential of an F_1 hybrid before undertaking extensive field trials.

Mitochondrial complementation is a more complex phenomenon, reported to occur when mitochondria isolated from the two prospective parents are mixed *in vitro*. The phenomenon can be recognized by the superior biochemical activity (measured by the rate of oxidation and efficiency of phosphorylation) of a 1 : 1 mixture of the mitochondria of the two potential parents over the arithmetic mean of the two preparations when assayed separately. Sarkissian and Srivastava⁷ claim that there is a correlation between mitochondrial complementation and mitochondrial heterosis in F_1 hybrid maize cultivars and have extended their observations to include F_1 hybrid wheat³. Mitochondrial complementation is therefore a potentially much more useful tool in plant breeding programmes than mitochondrial heterosis because it would predict, without first producing the hybrid, which cultivars, when crossed, would yield heterotic F_1 hybrids.

McDaniel⁶ reiterates that both mitochondrial heterosis and complementation can be used to predict the relative yield potentials of F_1 hybrid barley cultivars. He states that use of mitochondrial complementation "should provide a more efficient and rapid method of screening potential breeding material and evaluating hybrids". However, McDaniel does not discuss the relative merits of complementation and heterosis

to the plant breeder, nor does he distinguish between the phenomena of heterosis and complementation in his correlation of mitochondrial data with yield data and, therefore, does not provide convincing evidence that either phenomenon alone is correlated with yield potential.

Before incorporating mitochondrial complementation tests routinely into any breeding programme it is necessary to assess the technique for its reproducibility and reliability, and our investigation has had this as its primary objective. Initial tests were carried out using the barley cultivars 18-17 and Arivat, as McDaniel reported⁴ that mixtures of mitochondria obtained from these two cultivars gave an increase in ADP : O ratios of 22%. In a standard experiment we extracted mitochondria from twenty barley scutella with embryonic axes from seeds germinated for 18 h at 27° C. The tissue was homogenized in a chilled pestle and mortar for one minute in 5 ml. of isolating buffer. The resultant brei was filtered through nylon mesh and the filtrate centrifuged at 2,000*g* for two minutes to remove starch and cell debris. We sedimented the mitochondria by centrifugation at 30,000*g* for three minutes, and further separated the mitochondria from residual starch by pouring off the supernatant, adding 3 ml. of washing buffer, rotating the tube through 180° in the rotor, and centrifuging at 6,000*g* for one minute⁸. The mitochondrial pellet was then suspended in 0.1 ml. of assay buffer. The buffer solutions with which we isolated, washed and assayed the mitochondria were as described by McDaniel⁴ except that 0.1% of fatty acid free bovine serum albumin was added to all three. The rate of oxygen uptake and ADP : O ratios were measured using conventional polarographic techniques with a total assay volume of 0.3 to 0.5 ml. Using this method mitochondria were obtained with respiratory control ratios of between 3.0 and 5.0. Although the ADP : O ratios were low, they were of the same order as those quoted by McDaniel (Table 1).

Preliminary replicated control experiments indicated the need for a high degree of precision at every stage of the procedure as a prerequisite to reliable data, necessary if the tech-

nique is to be used in a large scale selection programme. The consistent production of uniform pellets of mitochondria was largely achieved by producing mitochondria from both parents and the 1 : 1 mixture simultaneously in one "preparation". The basic experimental design used was a 3 × 3 Latin square in which the mitochondria from each cultivar were assayed in each of the three "preparations". Within successive "preparations", the mitochondrial pellets of the three cultivars were assayed in rotation to eliminate bias due to ageing. No complementation could be detected using this technique (Table 1, experiment *B*). Many modifications were tried as listed in Table 1 but it was not possible to obtain any mitochondrial complementation statistically significant at the 5% level in either the ADP : O ratio or any other parameter of mitochondrial activity.

A similar series of experiments was carried out using the wheat cultivars 31 MS and 28, in which Sarkissian and Srivastava report³ finding levels of mitochondrial complementation of up to 54%. In our initial experiment *H* the technique described by Sarkissian and Srivastava³ was used, but in later experiments *I* to *L* the modifications listed in Table 2 were made as recommended by Sage and Hobson (private communication). It is apparent from data in Table 2 that under no conditions were we able to detect statistically significant levels of complementation.

Table 2 Statistical Analysis of Mitochondrial Complementation in Wheat Cultivars 31 MS and 28

Exp. No.	31 MS ADP : O Mean	1:1 Mix ADP : O Mean	28 ADP : O Mean	MC obtained	Sig. at 5% level	Least MC detectable at 5% level
<i>G</i>	3.8	5.4	3.2	+54.3%	—	—
<i>H</i>	1.81*	1.82*	1.78*	+1.3%	N.s.	±10.3%
<i>I</i>	2.07*	2.23†	2.23†	+3.9%	N.s.	±5.6%
<i>J</i>	2.28*	2.40*	2.41*	+2.3%	N.s.	±7.7%
<i>K</i>	2.15*	2.23*†	2.30†	+0.3%	N.s.	±5.6%
<i>L</i>	2.10*	2.03*	2.20*	-5.8%	N.s.	±8.4%

In experiments *G* and *H* the substrate was α -oxoglutarate (10 mM). In experiments *I*, *J*, *K* and *L* the substrate was α -oxoglutarate (10 mM) with malonate (5 mM).

Means of ADP : O with different (*, †) superscripts, within each experiment, are significantly different at the 5% level.

Mitochondrial complementation (MC) is calculated as given in Table 1.

Experiments *G* to *L* were carried out with the following modifications:

G, Sarkissian and Srivastava's data³.

H, The techniques described by Sarkissian and Srivastava³ were used in the context of a randomized double 3 × 3 Latin square.

I, The composition of the buffer solutions was modified as recommended by Sage and Hobson. The isolating and washing buffer comprised sucrose (0.45 M), BSA (0.075%), EGTA (2 mM), TES (1 mM), Mg Cl₂ (1 mM) and potassium phosphate (67 mM, pH 7.2), and the assay buffer was changed to mannitol (0.3 M), KCl (10 mM), Mg Cl₂ (5 mM), EGTA (2 mM), TES (10 mM), BSA (0.075%) and potassium phosphate (10 mM, pH 7.2).

J, The time of isolation of mitochondria was reduced from 15.0 min to 12.5 min.

K, The operators were given coded materials to eliminate personal bias.

L, The type of fabric used by Hobson for filtering the wheat shoot brei was used, and the concentration of mitochondrial protein in the assay buffer solution was raised from about 0.6 mg ml.⁻¹ to 0.75 mg ml.⁻¹. In Experiments *I* to *L* each genotype determination was replicated six times in individual preparations at random, as in *E*.

The reason for the discrepancy between this data and that reported from other laboratories cannot as yet be defined. The final column in Tables 1 and 2 quotes the minimum levels of mitochondrial complementation significant at the 5% level which could have been detected in our experiments, calculated from each respective error term. Clearly levels as large as those quoted by McDaniel, Sarkissian and Srivastava could be detected, as well as the low level of +9% found by Sage and Hobson with wheat cultivars Peko and Cappelle Desprez.

The divergence apparently lies in an undefined detail of

Table 1 Statistical Analysis of Mitochondrial Complementation in Barley Cultivars 18-17 and Arivat

Exp. No.	18-17 ADP : O mean	1:1 Mix ADP : O mean	Arivat ADP : O mean	MC obtained	Sig. at 5% level	Least MC detectable at 5% level
<i>A</i>	2.08*	2.44†	1.92*	+22.0%	—	—
<i>B</i>	1.61*	1.68*	1.70*	+1.5%	N.s.	±8.7%
<i>C</i>	1.47*	1.54*	1.62*	-0.6%	N.s.	±8.7%
<i>D</i>	1.84*	1.84*	2.00†	-4.5%	N.s.	±5.2%
<i>E</i>	1.23*	1.26*	1.39†	-4.3%	N.s.	±9.9%
<i>F</i>	1.62*	1.61*	1.72*	-3.7%	N.s.	±9.0%

The substrate was α -oxoglutarate (12 mM in experiments *B*, *C* and *D* and 10 mM in experiments *E* and *F*). Means of ADP : O ratios with different (*, †) superscripts, within each experiment, are significantly different at the 5% level. Mitochondrial complementation is calculated by the expression:

$$MC = \frac{ADP : O (1:1 \text{ mix}) - ADP : O (MP)}{ADP : O (MP)} \times 100$$

where the mid-parent value, ADP : O (MP), is derived from $\frac{1}{2}ADP : O (18-17) + \frac{1}{2}ADP : O (Arivat)$.

Experiments *A* to *F* were carried out with the following modifications:

A, McDaniel's data⁴.

B, The techniques described in the text were used.

C, The fast isolation procedure was replaced by a slow method, comprising spins of 1,000*g* (5 min) and two of 10,000*g* (each 10 min). Furthermore, BSA was omitted from all solutions, and NAD and TPP omitted from the assay buffer solution.

D, Seed samples of 18-17 and Arivat, used in experiment *A*, were obtained from McDaniel and analysed as in experiment *B*.

E, The germination time for the barley seedlings was increased to 30 h, and mitochondria isolated by the slow procedure. Six replicates of each genotype were prepared individually, thereby dispensing with the Latin square.

F, The 1 : 1 mixture was prepared from pellets of isolated mitochondria in a randomized double 3 × 3 Latin square, but otherwise the technique was as described in the text.

procedure, despite frequent and exhaustive discussions with each group of workers. One notable point of interest, however, is the relatively large differences in the ADP : O ratios, particularly with wheat, obtained by the different groups. The ADP : O ratios quoted in this paper (2.2) are lower than those obtained by Sarkissian and Srivastava (3.0–6.0)³ and Sage and Hobson (3.0). We suspect that further investigation of this discrepancy will eventually help to resolve the controversy concerning mitochondrial complementation.

The conclusion from our investigations is that we have been unable to demonstrate mitochondrial complementation, despite paying careful attention to published procedures. Until the phenomenon is better understood and the experimental conditions necessary for its expression more accurately described, we cannot, at present, recommend that mitochondrial complementation be used in a hybrid cereal breeding programme.

We thank the Rambar Corporation, Dr R. G. McDaniel and Dr R. T. Ramage for gifts of barley cultivars 18-17 and Arivat, and DeKalb AgResearch for wheat cultivars 31 MS and 28. We wish to acknowledge the CAPS award from the Science Research Council for C. J. Brunton.

J. R. S. ELLIS

*The Lord Rank Research Centre,
High Wycombe, Bucks*

C. J. BRUNTON
J. M. PALMER

*Department of Botany,
Imperial College,
University of London*

Received July 21, 1972.

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An Increasing Fitness Function for a Population with Many Niches

THE mean viability, V , increases from generation to generation^{1–3} for a single autosomal locus with multiple alleles. Ewens⁴ has recently proved that for an arbitrary number of linked loci, with viability additive over loci, mean viability is again increasing. This latter result was proved using the former.

Here I show a further case in which a fairly simple fitness function increases from generation to generation. Levene⁵ formulated a model in which there were a number, m , of niches. Mating was at random amongst the total population, the resultant offspring migrating to the niches, which were of relative size c_i , and being subject to selection in those niches.

In a population in which there are n alleles A_j ($j=1, 2, \dots, n$), and the viability of the $A_j A_k$ genotype in the i th niche is a_{ijk} , p_j is the frequency of the j th allele just before mating, and p_j' is the frequency one generation later, then⁶

$$p_j' = \frac{\sum_{i=1}^m \sum_{k=1}^n \frac{c_i a_{ijk} p_j p_k}{V_i}}$$

where $V_i = \sum_{j=1}^n \sum_{k=1}^n a_{ijk} p_j p_k$, the mean viability in the i th niche.

Writing $V = \sum_{i=1}^m V_i c_i$ we obtain

$$p_j' = \frac{p_j \partial V / \partial p_j}{\sum_{j=1}^n p_j \partial V / \partial p_j} \quad (1)$$

Equation (1) is essentially equivalent to that studied by Baum and Eagon⁷. They demonstrated that $V' \geq V$, with equality only at an equilibrium of the system, that is, when $p_j = p_j'$ for all j . Thus the fitness function V increases from generation to generation. In this case V is considerably more complicated than in the single niche problems, and the possibility arises of several equilibria or of continuous curves of equilibria existing⁶.

We should note that all the cases of increasing fitness mentioned above, and also the p -sex model case of Blakeley⁸ are direct, or indirect, consequences of the same theorem⁷. All these models have certain basic features, first, random mating amongst the whole population, and second, identical viabilities for the two sexes. The addition of extra complexity such as inbreeding, differential selection for males and females, or asymmetry of genetic system destroys the property of increasing V .

Finally we note that the various systems with increasing V can be combined; V will increase for selection with n alleles, m niches, k loci with additive fitness for loci, and p sexes.

C. CANNINGS

*Department of Probability and Statistics,
University of Sheffield,
Sheffield S3 7RH*

Received October 2, 1972.

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Depression of Yield in Ryegrass Exposed to Sulphur Dioxide

PREVIOUS work¹ has shown that substantial economic losses may occur when S23 ryegrass is grown in areas of high coal-smoke pollution. Evidence suggested that sulphur dioxide was the toxic factor producing the depression in yield, although this could not be proved. We decided to investigate the effects of prolonged periods of low SO₂ concentration on the growth of S23 *Lolium perenne* in order to establish whether SO₂ can cause invisible injury and also to measure the depression of yields at different concentrations of the gas. We also used clones of wild *L. perenne* collected from Helmsore in a polluted region of East Lancashire. Observations at the ADAS Experimental Husbandry Farm at Helmsore have indicated that the native ryegrass in the district is apparently tolerant to the polluted atmosphere (C. H. Mudd, personal communication).

We grew the plants in bowls inside two Perspex chambers situated in an open-sided greenhouse. The chambers were ventilated with the ambient air, which was purified by passage through activated charcoal, followed by an absolute filter. A controlled uniformly distributed concentration of sulphur dioxide was maintained in one chamber, by bleeding SO₂ at a controlled rate into the air inlet pipe. The plants were grown in a well fertilized brown earth, watered by sub-irrigation.

Table 1 Final Yield of S23 *Lolium perenne* Grown for Nine Weeks in 343 $\mu\text{g m}^{-3}$ or 14 $\mu\text{g m}^{-3}$ SO_2

	No. of tillers	No. of living leaves	No. of dead leaves	Dry weight living leaves, g	Dry weight "stubble", g	Dry weight dead leaves, g	Leaf area, cm^2
SO_2 plants	5.95 ± 0.66	19.59 ± 1.96	6.64 ± 0.18	0.175 ± 0.016	0.082 ± 0.008	0.042 ± 0.001	94.4 ± 9.0
Control plants	10.20 ± 0.33	34.02 ± 1.19	4.56 ± 0.23	0.324 ± 0.012	0.155 ± 0.008	0.023 ± 0.002	168.6 ± 4.6
Decrease in productivity of SO_2 plants below controls	41.7%	43.0%	45.6%	46.1%	46.9%	79.3%	44.0%
$P=$	<0.001	<0.001	increase over control 0.02–0.05	<0.001	<0.001	increase over control <0.001	<0.001

Means per plant with standard errors shown.

Nine bowls in each chamber were sown with S23 seed and thinned out to sixteen plants per bowl; six bowls per chamber were planted with sixteen uniform tillers from two Helmschore clones. The SO_2 concentration inside each chamber was monitored for weekly periods throughout the experiments, using the standard titration method² and the accuracy of the technique was checked by regular sulphate analyses of the hydrogen peroxide solution.

In the first experiment the S23 seeds were sown and the Helmschore tillers planted on 3.8.71. The experiment continued for nine weeks and the mean SO_2 concentration was 343 $\mu\text{g m}^{-3}$ (0.120 p.p.m.) in the polluted chamber and 14 $\mu\text{g m}^{-3}$ (0.005 p.p.m.) in the control. A non-destructive growth analysis was carried out after five weeks, when the numbers of leaves, numbers of tillers, and the length of the third leaf on each S23 plant were determined. This indicated decreases in yield of the plants exposed to the higher SO_2 concentrations, of 13% in leaf number, 22% in tiller number and 8% in the length of the third leaf.

At the termination of the experiment the S23 plants in the polluted chamber were much smaller than those in the control chamber; they were also rather chlorotic in comparison with the controls, which looked healthy. Living leaves, dead leaves and tillers were counted on each plant and the dry weights determined for living leaf blades, dead leaf blades and the remaining "stubble"; leaf areas were also determined.

A reduction in yield of about 46% occurred in the polluted chamber S23 plants in comparison with the S23 plants grown in the control chamber (Table 1). This was due to a decrease in leaf and tiller production and also by an increase in the rate of senescence of the leaves; this is in agreement with the findings of Bleasdale¹. The reduced number of leaves on the SO_2 plants was primarily the result of lower leaf production rather than the increased rate of senescence.

For the Helmschore plants no significant difference was found in the productivity of either clone between the two treatments, except that more dead leaves were present in the control chamber although the dry weight was the same in each case; they also showed a healthy green appearance in both chambers.

A second experiment was carried out with the role of the control and polluted chambers reversed to show that the depression in yield in the first experiment was not caused by slight differences between the chambers. The experiment was carried out over the winter with the mean SO_2 concentration

lowered to 191 $\mu\text{g m}^{-3}$ (0.067 p.p.m.) which is within the range of mean winter SO_2 levels reported from polluted rural areas³. The experiment commenced on 12.10.71 and terminated on the 10.4.72, a total of twenty-six weeks. Growth occurred slowly throughout most of the winter, but increased rapidly during the warm weather in March and early April.

Six weeks after sowing, when the plants were mostly at the 3-leaf stage, the productivity of the S23 was examined by measuring the length of the second leaf. This indicated a slightly but significantly better growth in the polluted chamber. Sixteen weeks from sowing another non-destructive growth analysis was carried out on the S23 plants, counting numbers of living leaves, dead leaves and tillers. The results indicated a depression in plants exposed to SO_2 of 27% in living leaf number and 41% in tiller number and an increase of 75% in the number of dead leaves.

The results of the final harvest are shown in Table 2. The dry weight of the shoots of the S23 plants in the polluted chamber was depressed by 52% in comparison with the controls, this being accounted for by a depression in leaf and tiller formation and an increased rate of leaf senescence. No significant difference was found between treatments in the dry weights of the shoots of the Helmschore plants.

Many aspects of the problem have still to be investigated. Differences in wind speed may have an effect in that a higher speed will decrease r_a , the aerodynamic resistance, and hence increase the dose of SO_2 to the surface of the plant; plants growing under field conditions with higher wind speeds than in the experimental chambers might react in a different manner to SO_2 pollution, as a result of the combined effects of wind and SO_2 . The results indicate that SO_2 depresses yields under both summer and winter conditions, but further investigations are necessary to determine the relative importance of SO_2 in the ambient air at different seasons. Another important factor is the possible ameliorating effect on SO_2 damage of increased levels of nitrogen fertilizers, which will be investigated in the next stage of this work. In spite of the limited extent of this preliminary work, we feel that the results so far suggest that SO_2 concentrations typical of polluted districts can seriously depress the yield of S23 *Lolium perenne*. It is possible that the native population in polluted districts has been selected for tolerance to SO_2 pollution and this opens up possibilities for breeding tolerance into commercially available varieties. It is difficult to say whether or not invisible injury has occurred; the polluted plants were somewhat yellow, but this

Table 2 Final Yield of S23 *Lolium perenne* Grown for Twenty-six Weeks in 191 $\mu\text{g m}^{-3}$ or 9 $\mu\text{g m}^{-3}$ SO_2

	No. of tillers	No. of living leaves	No. of dead leaves	Dry weight living leaves, g	Dry weight "stubble", g	Dry weight dead leaves, g	Leaf area, cm^2
SO_2 plants	14.84 ± 0.67	47.31 ± 2.31	12.02 ± 0.55	0.388 ± 0.021	0.217 ± 0.014	0.047 ± 0.003	203.6 ± 13.2
Control plants	25.18 ± 0.77	85.61 ± 2.35	6.39 ± 0.39	0.791 ± 0.026	0.478 ± 0.019	0.027 ± 0.003	417.2 ± 18.0
Decrease in productivity of SO_2 plants below controls	41.1%	44.7%	88.1%	50.9%	54.6%	77.7%	51.2%
$P=$	<0.001	<0.001	increase over control <0.001	<0.001	<0.001	increase over control <0.001	<0.001

Means per plant with standard errors shown.

might not be noticed in the field in the absence of an adjacent control.

We thank Professor A. J. Rutter for the use of facilities.

J. N. B. BELL

Department of Botany,
Imperial College Field Station,
Silwood Park,
Ascot

W. S. CLOUGH

Health Physics and Medical Division,
AERE Harwell

Received July 17; revised July 26, 1972.

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Crystallization *in vivo* of Rhabdophane in Human Lungs

We have observed an unusual accumulation of inorganic rod-like particles with a mean diameter of about 10 nm in the lungs of two reproduction photographers (Fig. 1). From the electron diffraction of the particles the *d*-spacings were calculated (Table 1).

The X-ray powder diffraction pattern of the isolated lung dust does not show any additional lines except those of quartz which can be traced in every human lung. A semiquantitative X-ray fluorescence analysis showed the presence of the following elements (in order of decreasing frequency): cerium, lanthanum, calcium, neodymium, thorium, praseodymium, yttrium and samarium. In addition; silicon, iron and potassium, which are common in lung tissue, were also detected. Peaks at 1,060 cm⁻¹, 623 cm⁻¹ and 540 cm⁻¹ in the infrared spectrum indicate that the rare earth elements are combined with phosphate ions.

These results leave no doubt that the particles consist of rhabdophane, (La, Ce, Nd . . .) PO₄·H₂O, or at least partly of the rather rare thorium-rich variety brockite. The analysis of the elements contained in the ash of a arc-lamp electrode which had been used in the workshop where both photographers

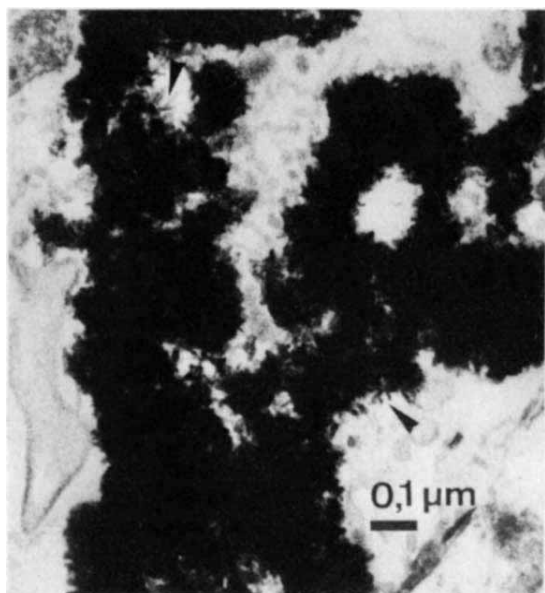


Fig. 1 Electron micrograph of lung tissue: detail of a rhabdophane containing cellular inclusion body.

Table 1 Electron Diffraction Pattern of Lung Dust and *d*-Spacings of Rhabdophane and Brockite

Lung dust		Rhabdophane (ASTM 12-277)		Brockite (ASTM 15-248)		
<i>d</i> (Å)	<i>I</i> / <i>I</i> ₀	<i>d</i> (Å)	<i>I</i> / <i>I</i> ₀	<i>d</i> (Å)	<i>I</i> / <i>I</i> ₀	<i>hkl</i>
6.08	30	6.07	60	6.06	40	100
4.39	60	4.40	80	4.37	70	101
3.53	50	3.49	60	3.47	50	110
3.04	90	3.02	100	3.03	100	200
2.82	100	2.83	80	2.83	70	102
2.36	20	2.36	40	2.37	30	112
2.12	40	2.15	80	2.15	70	211
1.93	20	1.92	40	1.92	30	301
1.86	40	1.859	60	1.86	50	212
Hexagonal		Hexagonal		Hexagonal		
<i>a</i> ₀ = 7.02		<i>a</i> ₀ = 6.98		<i>a</i> ₀ = 6.98		
<i>c</i> ₀ = 6.35 ₅		<i>c</i> ₀ = 6.39		<i>c</i> ₀ = 6.40		

had worked showed a pattern similar to the lung dust with respect to the rare earth elements. X-ray powder diffraction analysis revealed, however, that in the ash these elements were present as oxides. Therefore, it must be concluded that the inhaled oxides were gradually converted to rhabdophane (or brockite) within the lung tissue. To our knowledge the *in vivo* crystallization of these rare minerals has not been previously observed.

H. STICHER

Institute of Agricultural Chemistry,
Swiss Federal Institute of Technology,
Zürich

M. A. SPYCHER
J. R. RUETTNER

Institute of Pathological Anatomy,
University of Zürich

Dimethylnitrosamine in the Human Vaginal Vault

THE high frequency of cancer of the uterine cervix in southern Africans¹ and the general epidemiological features of the disease² suggest that chemical carcinogens might be found in infected discharge from the human cervix.

Pathological cervical and vaginal discharges contain cellular matter in various stages of degradation in a "protected" environment at pH (about 5.5), and temperature favourable for chemical change. Discharges are usually infected with a wide variety of microorganisms, often including *Trichomonas*, *Monilia* and *E. coli*. Nitrosamines, well known potent carcinogens³, might be synthesized by such organisms or formed when and where amines and nitrites (or possibly nitrates in reducing conditions) are present.

Samples were prepared and dimethylnitrosamine isolated by the technique of Nunn and Du Plessis⁴. Isolation and identification of nitrosamines in biological samples (especially when present in the p.p.b. range), based on final gas chromatograph-mass spectrometry, are the most reliable (L. S. Du P. and J. R. N., in preparation). Our identification of dimethylnitrosamine was based both on gas chromatography (where a peak coincided with that of dimethylnitrosamine under two different sets of conditions) and on the mass spectrum of the component responsible for this peak. This spectrum displayed distinctive peaks at mass 74 (parent ion of dimethylnitrosamine) and at mass 30, which could only have been due to NO⁺, characteristic of nitrosamines. Although the increased intensity of mass 30 could have been due to an ion such as ¹⁵N₂⁺, it is extremely unlikely here.

A detailed examination was made of pooled discharges taken from 100 African patients attending an antenatal clinic in Johannesburg. Specimens were collected at random, the criterion of choice being only the availability of material. Most patients in this first series were pregnant, because the

physiological (and pathological) secretion from the endocervix is quite profuse. Specimens were taken almost exclusively from patients at their first visit. Apart from overt bleeding, cases with cervical pathology were not excluded, but these probably accounted for only a very small proportion of those examined. Most could be termed "physiologically normal" cases.

Samples (about 0.2 to 0.5 ml. from each patient) were taken by bulb and Pasteur pipette. A sterile 'Tampax' outer insertion tube (the cardboard outer sleeve) was inserted into the axis of the vagina and the posterior vaginal wall was visualized. The pipette was then inserted, the contents of the posterior fornix aspirated by gentle suction from the bulb, and the tube discarded. Empty pipettes were used as control checks and during analysis were treated identically to the test pipettes. Each pipette was placed in a clean glass tube, which was sealed with a stopper and stored at low temperature. Distilled water (4 ml.) was added to each of the control and test collection tubes; the test ones were manipulated to ensure proper dispersal of the discharge material from the pipette into the water. The water in each test and control tube was then frozen by gentle mixing of the tube while immersed in liquid air. Tubes (without any grease on ground joints) were then freeze-dried. The distillate (about 30 ml.) was collected in a single collection flask and extracted with dichloromethane (4 × 3 ml.) after thawing. The organic phase was separated each time by pipette. The combined dichloromethane extracts (about 50 ml.) from the distillation of 36 tubes (100 test samples) were dried over $\text{Mg}(\text{ClO}_4)_2$ and concentrated to about 300 μl . in a flask fitted to a vacuum jacketed Vigreux column.

Chromatography of this solution revealed a number of peaks, one of which was coincident with that of dimethylnitrosamine (retention time 3.2 min). No peaks were detected when control materials were analysed by identical gas chromatography; the test extract was therefore further examined in a gas chromatograph-mass spectrometer linked system ('Pye-Unicam' gas chromatograph and an 'A.E.I. MS30' mass spectrometer) under carefully specified conditions.

One of the peaks in the gas chromatogram (shown by the total ion monitor) again coincided exactly with that given by dimethylnitrosamine (retention time 68 s) under the same conditions. The mass spectrometer was set to scan at 10 s per decade. Blank—that is, "background"—mass spectra were recorded at the retention time of dimethylnitrosamine after the injection of pure dichloromethane into the gas chromatograph. A series of mass spectra was recorded across the peak in question (25 μl . injection of the dichloromethane extract). The maximum intensity of mass 74 was 148 units, as compared with a background intensity of 12 units, a twelve-fold increase. Concurrently, mass 30 (NO^+) doubled in intensity from 11 to 22 units.

We suggest these results indicate the probable presence of dimethylnitrosamine in pooled specimens of human cervical and vaginal discharge matter; no attempt has been made to estimate the actual amounts present.

We thank the Anglo American Corporation of South Africa and the National Cancer Association of South Africa for support.

J. S. HARINGTON

Cancer Research Unit,
National Cancer Association of South Africa,
South African Institute for Medical Research,
P.O. Box 1038,
Johannesburg

J. R. NUNN

Department of Chemistry,
Rhodes University,
Grahamstown

L. IRWIG

Department of Clinical Epidemiology and Social Medicine,
St Thomas's Hospital Medical School,
London

Received July 25; revised September 22, 1972.

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In vitro Fertilization of Rat Eggs

AFTER the recognition of "capacitation of sperm" by Austin¹ and Chang², successful *in vitro* fertilization of mammalian eggs was achieved in many species³⁻¹⁷. Although fertilization of rat eggs *in vitro* has been attempted^{1,18-20}, incorporation of sperm into the vitellus was observed only after the dissolution of zona pellucida by chymotrypsin¹⁹. Here we report fertilization of intact rat eggs *in vitro* and the results obtained.

Mature female CD strain rats were kept in a constant temperature room (23–25° C) with artificial light (19.00–7.00 h darkness) and their vaginal smears inspected daily. As ovulation usually occurs at about 2.00 h on the pro-oestrous to oestrous night²¹, the eggs were considered to be about 4 to 5 h and 7.5 to 8.5 h after ovulation when pro-oestrous rats were killed at 6.00 to 7.00 and 9.30 to 10.30 h respectively on the next day. Superovulation of mature rats was performed by

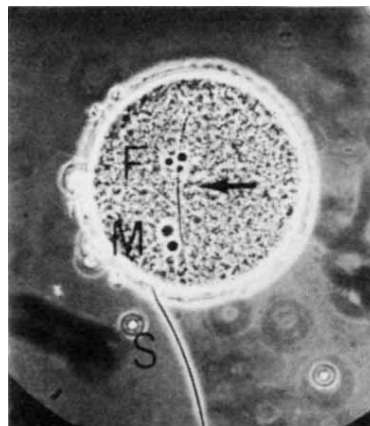


Fig. 1 A rat egg at pronuclear stage, fertilized *in vitro*, showing male pronucleus (M), female pronucleus (F), the fertilizing sperm tail (arrow) and a sperm attached to the zona pellucida (S). Photographed before fixation under a phase-contrast microscope. Sperm for insemination were prepared either from the cauda epididymis or from the uterus of a female mated 0.5 to 1 h, or 10 to 11 h, previously. A drop of dense sperm mass was put into a watch glass and covered with 3 ml. of medium (modified Krebs-Ringer bicarbonate solution containing 114.2 mM NaCl, 4.78 mM KCl, 1.71 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.19 mM KH_2PO_4 , 1.19 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 25.07 mM NaHCO_3 , 0.55 mM sodium pyruvate, 21.58 mM sodium lactate and 5.55 mM glucose, to which 4 mg ml^{-1} of crystalline bovine serum albumin, 50 $\mu\text{g} \text{ml}^{-1}$ of streptomycin sulphate and 75 $\mu\text{g} \text{ml}^{-1}$ of penicillin G (potassium salt) were added). The pH value of the medium was adjusted to 7.4 to 7.5 by addition of 1 M NaOH and the final solution was filtered through a millipore filter to ensure asepsis. Blood of female rats was collected by heart puncture, allowed to clot and then centrifuged. Serum was sterilized by filtration through a millipore filter and stored under paraffin oil at 2 to 5° C for no longer than five days. The uterine fluid was collected from oestrous rats by means of a syringe needle. Insemination was performed by adding 0.4 ml. of the sperm suspension to the egg clot under the oil, after removing oviducts and debris. In some cases the egg clot was washed three times with the same medium to remove the oviducal fluid. After adding 0.1 or 0.2 ml. of heated rat serum (at 56° C for 40 min), the eggs and sperm were thoroughly mixed and incubated at 37° C in an atmosphere of 5% CO_2 in air. The final concentration of sperm in the suspension was 1,000 to 4,000 sperm mm^{-3} .

i.p. injection of 30 IU of pregnant mare's serum (PMS) on the morning of oestrus and 30 IU of human chorionic gonadotrophin (HCG), 52 to 58 h later; the rats were killed 14 to 16 h after injection of HCG. Oviducts were placed under light paraffin oil, equilibrated with 5% CO₂ in air in the presence of a small volume of saline, in a watch glass kept at 37° C and the eggs in cumulus clot were released by dissecting the ampular portion of the oviducts. Sperm for insemination were prepared. The eggs were inseminated with sperm prepared as in Fig. 1.

After incubation for 8 to 12 h, the eggs were removed, mounted on a slide and examined. They were then fixed with neutral formalin and stained for the assessment of fertilization. The eggs which had sperm within their perivitelline space (supplementary sperm) were defined as "penetrated" eggs. When these eggs had either enlarged sperm head(s) or male pronucleus (ei) with fertilizing sperm tail (s) in or on the vitellus, they were considered as undergoing fertilization.

containing rat serum and uterine fluid, were used, they remained motile for longer than uterine-incubated sperm. This, and the higher proportion of eggs penetrated by sperm recovered from the uterus 10 to 11 h after mating demonstrated clearly that rat sperm do need capacitation in the female tract before they are capable of penetrating the egg. Hamster^{6,8}, mouse¹⁵, guinea pig¹⁷ and perhaps human^{9,10} sperm can be capacitated *in vitro*, but we have shown the difficulty of capacitating rat sperm *in vitro*. Rat sperm is similar to rabbit sperm in this respect.

Of 126 eggs undergoing fertilization, 114 (90%) were monospermic and 12 (10%) were polyspermic; 12 had an enlarged sperm head and 114 had at least one male pronucleus. Fifty monospermic eggs and 12 polyspermic eggs had 1 to 26 supplementary sperm. Supplementary sperm (1 to 11) were also found in 88 unfertilized eggs, which indicates the occurrence of a vitelline block to sperm penetration as the eggs deteriorated *in vitro*. The incidence of polyspermy of rat eggs

Table 1 *In vitro* Fertilization of Rat Eggs

Mature female rats	Age of eggs (h after ovulation)	Sperm used	Medium used (medium : serum)	No. of females used	No. of eggs examined	No. of penetrated eggs (%)	No. of eggs undergoing fertilization (%)	No. of polyspermic eggs (%)
Naturally-ovulated	4-5	Uterine 0.5-1 h after mating	4 : 1	5	54	4 (7)	2 (4)	0
	4-5	Uterine 10-11 h after mating	4 : 0	5	56	6 (11)	4 (7)	1 (25)
			2 : 1	10	84	38 (45)	22 (26)	0
	4-5	Uterine 10-11 h after mating	4 : 1	5	55	25 (45)	25 (45)	9 (36)
	4-5*	Uterine 10-11 h after mating	2 : 1	5	54	13 (24)	10 (19)	1 (10)
	7.5-8.5	Uterine 10-11 h after mating	2 : 1	6	78	1 (1)	1 (1)	0
	7.5-8.5	Uterine 10-11 h after mating	4 : 1	6	74	4 (5)	3 (4)	0
	4-5	Epididymal †	With serum and uterine fluid	5	52	0	0	0
Super-ovulated	1-3	Uterine 0.5-1 h after mating	4 : 1	12	280	19 (7)	4 (1)	0
	1-3	Uterine 10-11 h after mating	2 : 1	13	363	62 (17)	30 (8)	1 (3)
	1-3	Uterine 10-11 h after mating	4 : 1	4	101	22 (22)	13 (13)	1 (8)
	1-3*	Uterine 10-11 h after mating	2 : 1	5	136	12 (9)	8 (6)	0
	1-3*	Uterine 10-11 h after mating	4 : 1	4	133	14 (11)	8 (6)	0
	1-3	Epididymal †	With serum and uterine fluid	8	229	0	0	0

* Egg clot was washed three times before insemination.

† Epididymal sperm were incubated in the presence of uterine fluid for 2-7 h before insemination.

Fig. 1 shows a rat egg fertilized *in vitro* at the pronuclear stage. Table 1 shows that the eggs recovered from naturally-ovulated rats have a better chance ($P < 0.01$) of fertilization *in vitro* than those recovered from superovulated rats (highest penetration rates: 45% compared with 22%). The proportion of penetrated eggs was higher ($P < 0.05$) following insemination with sperm recovered from the uterus 10 to 11 h (17-45%) than 0.5 to 1 h after mating (7%) for both the naturally-ovulated and superovulated eggs. The eggs recovered 4 to 5 h after ovulation appeared to have a better chance of penetration (24-45%) than those recovered 7.5 to 8.5 h after ovulation (1-5%). By washing the egg clot before insemination, the proportion of penetration was decreased for both the naturally-ovulated (24% compared with 45%) and super-ovulated eggs (9-11 compared with 17-22%, not statistically significant), indicating that some beneficial factor may have been removed by washing. Although there was no obvious difference when different proportions of rat serum were added in the medium, sperm penetration was not observed when whole rat serum was used in a few experiments. Sperm penetration, however, was not observed in 52 naturally-ovulated eggs and 229 superovulated eggs when epididymal sperm, which had been pre-incubated for 2 to 7 h in the medium

fertilized *in vivo* is about 1.2%²². The *in vitro* incidence of polyspermy is comparatively high as in the case of hamster⁶ and mouse eggs¹⁵. Although fertilization of rat eggs *in vitro* was reported recently by Bregulla²⁰, the method used, the photographs published, and the high frequency of cleavage of unfertilized rat eggs *in vivo*²³, make his claim unconvincing.

This work was supported by grants from the US Public Health Service and the Ford Foundation. We thank Mrs Rose Bartke for assistance.

H. MIYAMOTO
M. C. CHANG

Worcester Foundation for Experimental Biology,
222 Maple Avenue,
Shrewsbury, Massachusetts 01545

Received June 19; revised August 8, 1972.

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Receptor—Bipolar Connectivity Patterns in Fish Retina

As well as rods, there are generally three or four morphological classes of cone cells in the retinae of non-mammalian vertebrates. They are commonly distinguished as single or double cones, and they differ in other more subtle anatomical respects. Microspectrophotometric analysis of the goldfish retina¹ suggested that the red and green photopic visual pigments are located in the separate elements of the twin cones in this species, and the blue one in the single cones. More recently², in the frog, it has been confirmed that the different pigments are segregated into the different retinal receptor types.

We have attempted to identify the chromatic receptors of the rudd (*Scardinius erythrophthalmus*, a common European cyprinid), and to observe, from their selective neural connexions with the bipolar population, something of the way in which wavelength-specific information is conveyed between the two synaptic layers of the retina.

Besides numerous rods, the rudd retina contains the quite distinct principal and accessory elements of double cones and single cones. These three cone classes are sometimes present in nearly equal numbers, most usually in a rather disorderly mosaic arrangement. In the light-adapted retina, they are slightly displaced from one another in that order in depth, with the principal elements of the double cones furthest from the lens of the eye, and the single cones nearest to it. There is good behavioural evidence that the rudd has a photopic trichromacy like that of the goldfish³, and it might be, as has been suggested for another species⁴, that the three pigments are appropriately ordered in depth among the different cone populations to compensate for chromatic aberration in the optics of the eye. Even in the eyes of small rudd, however, the axial dispersion of red and blue light is much greater than the axial separation of the cone types. There is an additional class of tiny single cones, sparsely though regularly distributed in the retina, but their properties are beyond the scope of this account.

Lacking a microspectrophotometer, we attempted to attribute the three colour mechanisms to one or other of the three major cone types by making colour separation photographs of isolated retinae⁵ that had been stripped of their rods. The tips of the cone outer segments were isolated in thin optical section, using the Leitz $\times 100$ apochromat oil immersion objective, and recorded, by transmitted light at 430, 530 and 625 nm, on Kodak 2745 film, both before and after bleaching. The negatives were analysed for cone transmission changes at the three wavelengths with a recording

microdensitometer. In good retinal preparations, the cones could be identified by their positions in depth and in the tangential receptor mosaic.

In this way, we have obtained evidence for a green-labile pigment in the accessory cones, and for a blue one in the single cones. At present it can only be argued by exclusion that the red pigment is located in the principal cones, for they have proved more difficult to register and identify, possibly because, being more distally placed in the retina, they are most likely to be distorted out of axial view. Ordering of the pigments between the cones is consistent with the sequence, if not the extent, of chromatic dispersion in the eye.

Having to that extent identified the different colour cones, we examined Golgi-impregnated retinae to establish the way in which the bipolar cells make selective contact with them in the outer plexiform layer. At the same time, it seemed important to relate such regularities as we found to other morphological distinctions between the bipolars. Although recent studies have tended to simplify this aspect of retinal anatomy, Cajal's drawings of lower vertebrate retinae⁶ show rather a large number of bipolar variants in each, distinguished by, among other features, the levels to which they address their synaptic terminals among the stratified amacrine and ganglion cell arborizations of the inner plexiform layer.

Table 1 Bipolar Types Classified According to their Connectivity in the Two Plexiform Layers of the Retina

	Receptive field diameter	Inner plexiform terminal strata	Receptor input (approximate numbers refer to cone types only)	Parthe's nomenclature
A	30 μm , 0.5°	6	Rods and principal (4) cones	Small rod bipolars
B	60 μm , 1.0°	6	Rods and principal (10) cones	Large rod bipolars
C	20 μm , 0.3°	1	Rods, principal (4) and accessory (4) cones	Small cone bipolars
	20 μm , 0.3°	2	Rods, principal (4) and accessory (4) cones	
D	75 μm , 1.25°	1, 4, 6	Single (15) cones	Large cone bipolars
	90 μm , 1.5°	4, 5	Accessory (25) cones	
E	40 μm , 0.6°	4, 6	Rods, principal (8) and accessory (8) cones	—

Receptive field dimensions in column 1 are approximate, and converted into angular subtense on the basis of the size of the fish used. Column 2 gives the strata in which the terminal swellings are located, and the system of their numbering is shown in Fig. 1a. Receptor input is specified in column 3, with approximate numbers in the case of the cones, and Parthe's⁷ nomenclature is given in the right hand column.

In the rudd, the inner plexiform is organized into six strata, which are often clearly visible by phase contrast in the osmium-dichromate fixed material used for Golgi-impregnation. Therefore it has been possible, in well oriented radial retinal slices, to specify the strata in which particular silvered bipolars terminate, before going on to characterize their outer plexiform connectivity. We have distinguished nine such bipolar types, and have determined the outer plexiform connectivity patterns of seven of them in the following way.

Tangential thin sections for electron microscopy were taken from the layer of rod and cone terminals at the distal margin of the outer plexiform, to locate those that were innervated by the silvered dendritic processes of particular named bipolars. The only criterion for synaptic relationship was that the processes be seen clearly to invaginate the single central cavities of the receptor terminals, and no account was taken of apposed membrane specializations or of proximity to terminal synaptic bars. The terminals of the different cones are difficult to distinguish from one another directly, but since the axons of the three major cone types preserve their

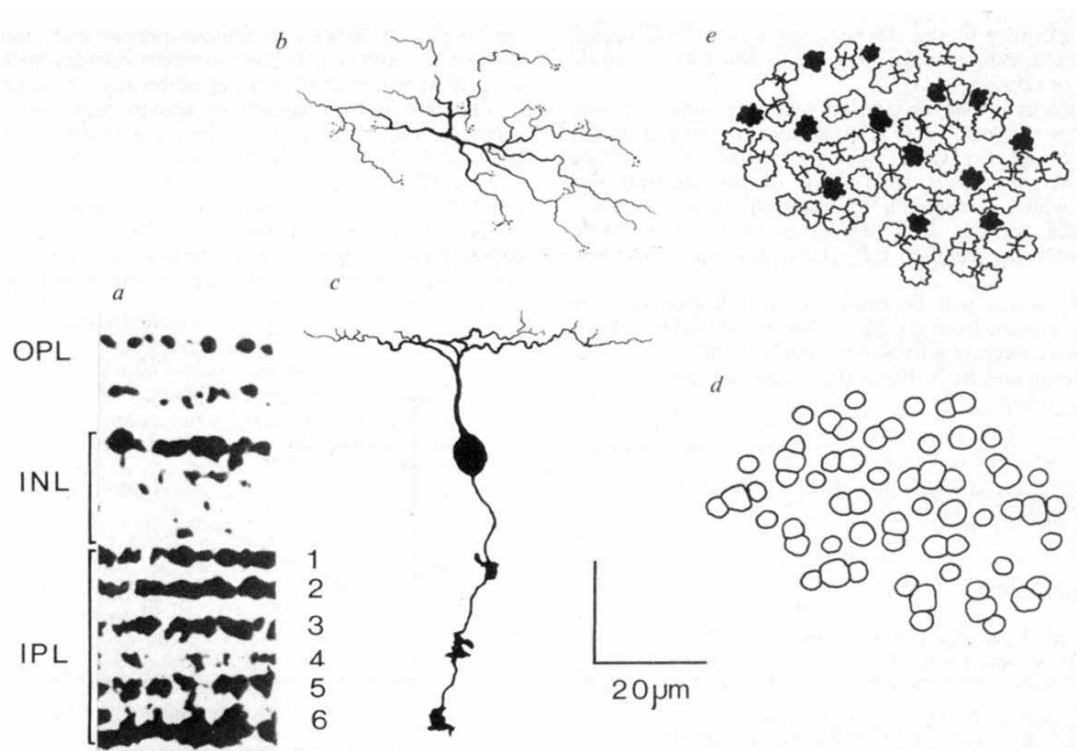


Fig. 1 The synaptic arrangements of a bipolar specific for single cones. *a*, Phase contrast micrograph of a thick radial slice of the retina to show the six strata of the inner plexiform layer (IPL) and the limits of the outer plexiform (OPL) and inner nuclear layer (INL). *b* and *c*, Camera lucida sketches, made with a $\times 100$ objective, of the tangential and radial profiles, respectively, of the Golgi-impregnated bipolar. The terminal synaptic swellings in the inner plexiform layer (*c*) were in register with strata 1, 4 and 6. Their tangential ramifications were incompletely impregnated. *d*, Tracing from light micrographs made at the level of the cone inner segments in the retinal area overlying the bipolar cell. Single cone locations are indicated by the smaller circles, and the double cones and several anomalous single principal elements and triples by larger circles or combinations of them. *e*, Tracing from tangential plane electron micrographs of the layer of cone terminals. Terminals that received silvered synaptic processes from the bipolar dendritic tree are filled black, and can be identified as belonging to single cones by comparing their layout with *d*. The pairing relations of double and triple cone terminals are indicated by short bars. In both *d* and *e* the rare fourth cone type (see text) is omitted from the maps. The calibration bars apply to all parts of the figure.

neighbour relations as they course through the outer nuclear layer, they can confidently be identified by comparing their arrangement in the EM sections with the locations of the various cones distal to the external limiting membrane, established by sectioning at this level for light microscopy.

Fig. 1 illustrates the outcome of this procedure for a bipolar that has terminal swellings in three of the inner plexiform strata, and connects exclusively to the pedicles of single cones in the outer plexiform layer. Table 1 summarizes the principal features of the bipolar types we have studied. From their general morphology, it has been possible, with one exception, to identify them with one or other of four bipolar categories recognized by Parthe⁷ in the fish retina, and we confirm the clarity of the distinctions made in that work. But for the rudd at least, some of the category names are inappropriate in the receptor connectivities they impute (Table 1), and some of them must be sub-divided to accommodate differences in the synaptic arrangements of the bipolars in the two plexiform layers.

Some functional aspects of this bipolar taxonomy deserve brief comment. First, in view of the classical distinction between the rod and cone bipolar systems^{6,7}, it has been surprising to find such widespread convergence of the two receptor types onto common bipolar cells. In fact, Stell⁸ has shown that Cajal's⁶ rod bipolars in the goldfish retina receive also cone input. In the rudd, this is true for both of the quite distinct varieties of these neurones (Table 1, *A* and *B*), and both of them draw their cone input exclusively from

the principal cones, which we take to constitute the red mechanism. Further, there is rod input to those small field bipolar cells that we equate with a class previously designated exclusive to cones⁷ (Table 1, *C*).

Both principal and accessory cones converge onto these small bipolars (Table 1, *C*), which are probably relatively numerous. Recordings from fish retinal ganglion cells have shown⁹ that there is widespread opponent interaction between the red and green cone channels, both within spatially antagonistic receptive field regions, and between them. There is no evidence at all of additive interaction between these receptor systems, and our anatomical finding therefore suggests that the red-green opponency may arise peripherally in the opposite synaptic effects of the two cone types on the small bipolars, rather than by interaction between bipolars in the inner plexiform layer.

There is some circumstantial support for this suggestion in recent ganglion cell recordings^{10,11}, which have shown that, just as the principal-accessory small field bipolars receive rod input (Table 1, *C*), so the red-green opponent units are driven by the rods when they are adequately dark adapted. The sign of the response mediated by the rods (on or off-discharge) is always the same as that of the red mechanism in light adaptation, and opposite therefore to green. It is interesting that the outer plexiform connectivity of the classical rod bipolars (Table 1, *A* and *B*) is consistent with this basic synergy of the red cones and the rods. The principal cone and rod inputs to these neurones presumably operate quite

separately according to the adaptational state of the retina, and it would therefore make little sense for their synaptic effects to be of opposite sign.

A final curiosity is that the bipolars that selectively innervate just one colour channel (Table 1, *D*) perform the widest spatial summation of all the types we have encountered. Their receptive fields are larger than those of the classical rod bipolars, of which one would, by tradition, expect the least acuity. Oddly enough, they are larger than the dendritic fields of at least one ganglion cell class in the inner plexiform layer.

This work, which will be described in full elsewhere, is supported by a grant from the SRC. We thank John Hall for introducing us to electron microscopy, and D. P. M. Northmore, W. R. A. Muntz and B. B. Boycott for their criticisms.

JOHN SCHOLLES
JACQUELINE MORRIS

Laboratory of Experimental Psychology,
University of Sussex,
Brighton

Received August 4, 1972.

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Phosphodiesterase in Tongue Epithelium: Activation by Bitter Taste Stimuli

THE role of cyclic nucleotides in stimulus-response coupling at the cellular level has received much attention¹. The level of adenosine cyclic 3',5'-monophosphate (cAMP) is dependent on its rate of synthesis, catalysed by adenylyl cyclase, and on its rate of destruction, catalysed by phosphodiesterase. Although most investigators have measured the regulation of cAMP levels by alterations in adenylyl cyclase activity, others have noted the presence of high levels of phosphodiesterase in certain sensory receptors and have suggested that this enzyme may be of special importance in such cells². We studied the phosphodiesterase activity in tongue epithelium to determine whether unusually high levels of this enzyme might also be associated with taste receptors, and to explore the possibility that taste stimuli might affect phosphodiesterase activity, thereby altering the intracellular cAMP concentrations.

Bovine tongues were obtained at a local slaughterhouse. The dorsal epithelium of the anterior portion of the tongue was dissected away from the underlying muscle, cut into small pieces, and frozen. Frozen pieces of epithelium were homogenized in a Waring Blender in 4 ml. of ice-cold 0.1 M phosphate buffer (pH 7.1) per g of tissue. Most of the insoluble material was collected by centrifugation (28,000g for 1 h at 3°C) and discarded. The supernatant fluid was fractionated with (NH₄)₂SO₄ and the fraction soluble in 20% (NH₄)₂SO₄, but not in 40% (NH₄)₂SO₄ was collected. This fraction was redissolved, dialysed against 1 mM MnCl₂, then centrifuged

for 10 min at 20,000g to remove precipitated material. The supernatant solution is the "extract" referred to below; the yield is approximately 150 mg of protein for every 100 g of tissue. No phosphodiesterase activity was detected in any fraction other than that precipitating between 20 and 40% (NH₄)₂SO₄, nor was any activity detected before dialysis against MnCl₂. This procedure is identical to that used in preparing an extract which, in the presence of sweet taste stimuli, undergoes changes in its ultraviolet spectrum³, and differs from a procedure described earlier^{4,5} only by the addition of the final dialysis step. Protein content was determined by the biuret reaction⁶.

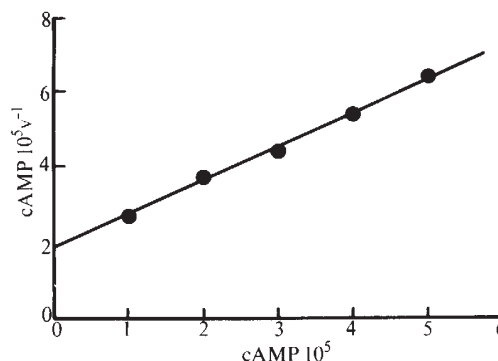
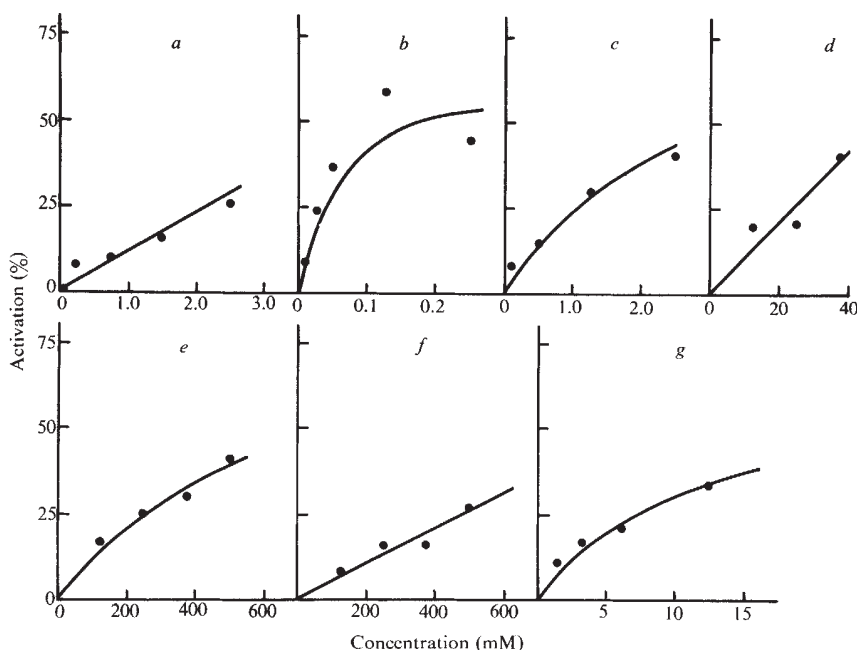


Fig. 1 Effect of substrate concentration on the hydrolysis of cAMP by the extract. Plotted according to the linear rearrangement of the Michaelis-Menten equation $S/v = S(1/V_m) + K_m/V_m$. The units of v are $\text{nmol min}^{-1} \text{mg}^{-1}$ of protein. Assayed at 30°C by the method of Drummond and Perrott-Yee¹⁰. The concentrations of the components of the reaction mixture were as follows: tris(hydroxymethyl)aminomethane, 75 mM; CaCl₂, 1 mM; MnCl₂, 1 mM; 5'-nucleotidase, 6.7 $\mu\text{g ml}^{-1}$ (0.08 units ml^{-1}); extract protein, 0.32 mg ml^{-1} ; adenosine deaminase, 0.17 $\mu\text{g ml}^{-1}$ (0.04 units ml^{-1}); cAMP, as indicated; pH 7.2.

The variation in the rate of hydrolysis of cAMP with varying cAMP concentrations is shown in Fig. 1. The behaviour is consistent with simple Michaelis-Menten kinetics, with a K_m of 2.0×10^{-5} M and a V_m of 1.1 nmol of substrate hydrolysed per min for every mg of protein. The values of K_m differed very little in different preparations, while V_m varied from 0.4 to 1.5 $\text{nmol min}^{-1} \text{mg}^{-1}$. These kinetic parameters are within the usual range encountered in mammalian tissue extracts⁷.

Preliminary experiments showed that some compounds which taste bitter resulted in increased phosphodiesterase activity when added to the extracts. Fischer and Griffin⁸ have published taste thresholds for a number of compounds, all determined under the same conditions and on persons selected as sensitive tasters, and their data were used to compare the potency of the compounds as taste stimulants with the effects of the compounds on phosphodiesterase activity. Seven structurally diverse compounds with widely varying taste thresholds were selected for study. The results are shown in Fig. 2. No attempt has been made to subject the concentration dependences to a theoretical analysis. Although there is considerable scatter in the points, the concentration of each compound which results in a 25% increase in the phosphodiesterase activity can be estimated with a reasonable degree of confidence. This concentration, which is referred to for brevity as " C_{25} ", is compared with taste thresholds in Fig. 3. The correlation is obvious. The straight line in Fig. 3 has a slope of 1.0, indicating that the potencies of the compounds as bitter taste stimuli are directly proportional to their potencies as activators of phosphodiesterase. The threshold concentration is usually about one-tenth the concentration which activates phosphodiesterase by 25%. The data cover a range of taste thresholds from 3×10^{-6} M to 1×10^{-1} M and suggest that the primary

Fig. 2 Activation of phosphodiesterase by bitter compounds. Activities were measured at 30° C by the method of Butcher and Sutherland¹¹. The composition of the reaction mixture was as described in Fig. 1, except that adenosine deaminase was omitted and the cAMP concentration was 1 mM. The reaction was stopped after 2 h of incubation by adding 0.5 ml. 1.2 M perchloric acid to 2.0 ml. reaction mixture, then determining P_i colorimetrically. Each point is an average of three experiments. *a*, Thioacetamide; *b*, quinine; *c*, propylthiouracil; *d*, phenylalanine; *e*, urea; *f*, acetamide; *g*, thiourea.



action of these compounds in stimulating bitter taste receptors is the activation of cyclic nucleotide phosphodiesterase. It is assumed that mammalian taste mechanisms are similar at the receptor level.

Imidazole, Mn^{2+} and Mg^{2+} are well known activators of phosphodiesterase⁷, and each tastes bitter. Strychnine and nicotine, both of which are bitter⁹, activate phosphodiesterase with values of C_{25} of approximately 7×10^{-5} M and 7×10^{-4} M, respectively. The only exception to the correlation of phosphodiesterase activation with bitterness which we have encountered is caffeine. Although bitter, caffeine is an inhibitor of phosphodiesterase from other tissues⁷, and inhibits the activity in our tongue extracts.

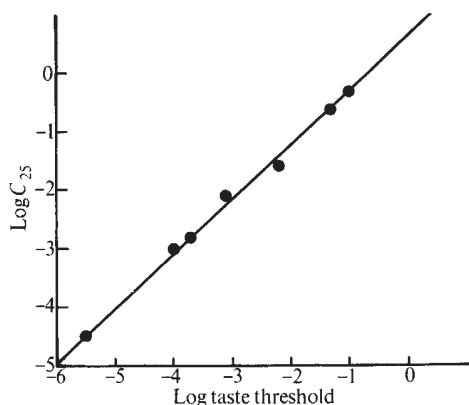


Fig. 3 The logarithms of C_{25} , determined from the data in Fig. 2, plotted against the logarithms of the taste thresholds reported by Fischer and Griffin⁸.

Our data suggest that reduction of cAMP level is involved in the excitation of bitter taste receptor cells, and that activation of phosphodiesterase is among the mechanisms by which bitter compounds cause this. Thus, an enzyme which is a ubiquitous component of mammalian tissues may play an integral role in the stimulation of bitter taste receptor cells. This is consistent with the report that taste buds do not appear to contain unique proteins¹².

I thank Miss Anne Byrd for technical assistance. This work

was supported by the National Institute of Dental Research, National Institutes of Health.

STEVEN PRICE

*Department of Physiology,
Medical College of Virginia,
Virginia Commonwealth University,
Richmond, Virginia 23219*

Received June 5; revised August 7, 1972.

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Inter-hemispheric Transfer of Meaningful Visual Information in Normal Human Subjects

IN an earlier study¹, normal human subjects made fewer recognition responses to complex random shapes² successively exposed in opposite visual fields than to those exposed twice in the same visual field. Subjects demonstrated superior recognition of shapes exposed twice in the same visual field even though vision was allowed in one eye during initial exposure and in the other during the recognition test. As

each visual field projects to the contralateral cerebral hemisphere, the results were interpreted as indicating that lateralized complex visual input does not automatically traverse the corpus callosum.

The two studies reported here were undertaken to determine whether stimulus association value affects visual recognition under conditions of successive exposure in the same or opposite visual field.

In the first experiment, 48 volunteers were randomly divided into two groups. None of the subjects wore glasses or had any other known visual defect. Sixteen trigrams were selected as stimuli; eight had an association value $\leq 25\%$ and eight had an association value $\geq 75\%$ ³. The eight high association value trigrams were: MSQ, TLW, BXT, PNK, HPD, CHF, JRN, ZBR; the eight low association value trigrams were: JBP, TZH, BFM, KXN, RGW, CSF, QWJ, FHL. The association value of these trigrams was determined by calculating the proportion of subjects in which each trigram elicited at least one meaningful associated thought or image. Each trigram was printed in black block letters 0.96 cm high on a white background and subtended 4° of visual angle. A 'Gerbrands' tachistoscope presented each trigram for 150 ms. Prior to trigram exposure, subjects focused on the point of intersection of a 2.54 cm black cross in the centre of the white pre-exposure field. The midpoints of the trigrams lay 5° to either the left or right of the pre-exposure fixation point.

The experiment was divided into initial exposure and recognition test periods. During initial exposure, twenty-four subjects were shown four low association value trigrams, two in the left visual field and two in the right visual field, each presented twice. For each subject, random procedures determined which four trigrams were to be shown and the order in which they were presented. Twelve subjects were tested with vision in their left eye blocked, twelve with vision in their right eye blocked. The other twenty-four subjects were shown four high association value trigrams under the same conditions.

Subjects were instructed to pay close attention to the trigrams because a recognition test would occur later. Approximately 2 s prior to each exposure a verbal "ready" signal was given. Subjects then fixated the point of intersection of the cross. To minimize the possibility of verbal mediation, subjects were instructed to count backwards from 100 in units of 3 as rapidly as possible for the duration of the exposure series. This technique has been successfully employed in studies of short term memory⁴. Approximately 10 s elapsed between the offset of a trigram and the onset of the next ready signal. Between 1 and 2 min after the end of the exposure series, each subject was again shown a series of trigrams and tested for recognition of those seen in the earlier series. The recognition test consisted of all eight trigrams, the four shown in the initial exposure series and four novel control trigrams, of either high or low association value. The trigrams were each exposed once, in random order, in one visual field under monocular viewing conditions. Half the subjects were tested for recognition with vision blocked in the same eye as during the exposure series and half with vision blocked in the opposite eye. Half the subjects tested with each eye had all eight trigrams exposed in the right visual field and half had the trigrams exposed in the left visual field. The experimental procedure was identical to that employed during the exposure series, with the exception that subjects were not required to count backwards. Subjects responded with a "yes" or "no" to indicate recognition or lack of it. Of the eight trigrams shown during the recognition test, two had been previously exposed in the same visual field, two had been previously exposed in the opposite visual field, and four had never before been presented.

For each subject in the low and high association value groups, the number of recognition ("yes") responses was calculated for trigrams previously exposed in the same visual field, those exposed in the opposite visual field and those never before presented (control trigrams).

These data are presented in percentage form in the upper portion of Table 1. An analysis of variance yielded the following results: (a) A significantly different number of recognition responses was made to the control, same field and changed field trigrams ($F=23.15$, $df=2/92$, $P<0.01$); and (b) a significant interaction between association value and trigram category (control, same visual field, changed visual field) was obtained ($F=3.64$, $df=2/92$, $P<0.05$). *t*-Tests performed on the interaction data yielded the following results: (a) For the low association value trigrams, those exposed twice in the same visual field were recognized more frequently than those exposed twice in opposite visual fields ($t=2.11$, $df=23$, $P<0.05$), though the latter were not recognized more frequently than the control trigrams ($t=1.77$, $df=23$, $P=n.s.$); (b) for the high association value trigrams, those exposed twice in the same visual field were not recognized more frequently than those exposed twice in opposite visual fields ($t=0.44$, $df=23$, $P=n.s.$), though the latter were recognized more frequently than the control trigrams ($t=7.44$, $df=23$, $P<0.01$).

Table 1 Percentage Recognition Responses to Control, Changed Field and Same Field Trigrams

Association value	Control	Trigrams Changed visual field	Same visual field
Experiment I (between-subject)			
Low ($N=24$)	34.37	50.00	72.91
High ($N=24$)	25.00	70.83	66.66
Experiment II (within-subject)			
Low ($N=48$)	38.02	54.17	73.96
High ($N=48$)	29.17	65.63	67.71

Contrary to expectation, stimuli exposed in the right visual field (to the left hemisphere) were recognized no more frequently than stimuli exposed in the left visual field (to the right hemisphere).

In the second experiment, another forty-eight visually normal volunteers were exposed to both high and low association value trigrams. Twenty-four subjects were first exposed to and tested on low association value trigrams and then, following a 2–3 min rest interval, exposed to and tested on high association value trigrams. The remaining twenty-four subjects received the sequence in reverse order. The procedures for selecting and presenting the trigrams during initial exposure and recognition test periods were identical with those used in the first experiment. The trigrams used in the second experiment were the same as those used in the first study. The results of the second study are presented in percentage form in the lower portion of Table 1. Analysis of variance yielded results identical with those obtained in the first experiment.

The lower portion of Table 1 shows that, while high association value changed field trigrams were recognized about as often as same field trigrams (65.63 vs 67.71%, respectively), low association value changed field trigrams were recognized less often than same field trigrams (54.17 vs 73.96%, respectively).

Collectively, the data from these two studies indicate that visual information of low association value, when projected to one cerebral hemisphere, does not readily traverse the corpus callosum. Visual information of high association value is apparently more readily transmitted between hemispheres.

Our results are in essential agreement with those reported by other investigators working with surgically prepared cats^{5,6}. Chiasma-sectioned cats were first taught monocular visual discriminations of varying degrees of difficulty. Following training, either the corpus callosum was sectioned or the "trained" visual cortex was ablated, and the "untrained" hemisphere was then tested for retention of the previously

learned discrimination. Retention by the untrained hemisphere was evident only for the easier discriminations.

This study was supported by a grant to K. M. K. from the Office of Research and Projects, Southern Illinois University, Edwardsville Campus. We thank Drs R. McLaughlin and D. Kohfeld for their helpful criticisms.

K. M. KLEINMAN
R. W. LITTLE

Department of Psychology,
Southern Illinois University,
Edwardsville, Illinois 62025

Received August 7; revised October 20, 1972.

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Long Term Effects on Immune Function of Early Nutritional Deprivation

SEVERE nutritional deficiency in early infancy arrests natural growth and maturation¹, resulting in depression of cell number and intellectual function of the brain in animals and man^{1,2}. In experimental animals, nutritional deprivation during gestation, or lactation results in permanent reduction in mature body size and organ weight with a decrease in metabolic efficiency³, even when optimum diets are fed after weaning. These effects are most severe when malnutrition occurs during both gestation and lactation; the most profound changes being in brain and thymus^{3,4}. A similar long term effect of early nutritional deprivation on the developing immunological system has been suggested to occur in man⁵.

We have examined the effect of a defined period of nutritional deprivation in infant mice at the time of weaning on the subsequent capacity of these animals to generate specific cellular and humoral immune responses to transplantation antigens. As infantile protein-calorie malnutrition in man is rarely found in the absence of infection, a further group of mice was subjected to intense antigenic stimulation during the

period of nutritional deprivation. All animals were returned to a full, normal diet after a 2-week deprivation period and were tested for their capacity to develop allogenic cellular cytotoxic and haemagglutinating responses between 5 and 12 weeks later.

Male C₃H₇/Umc low pathogen mice were weaned at 20 days of age and each litter distributed at random among four dietary groups. Normal protein diets were prepared according to the formula (g dry weight per 100 g diet): casein (N:1.5%) 30, mineral mixture USP XIV 4, vitamin mixture 2, agar 1 (Nutritional Biochemicals Corp.), dextrose 28, corn starch 28, corn oil 7. Low protein diets contained casein 6, and other components as in the full diet with corresponding increases in dextrose and corn starch. Both these diets were fed *ad libitum*, but exact quantities of each diet actually consumed were measured daily. Low-protein-low-calorie diets were prepared at twice the concentration of all ingredients in the low protein diet, with corresponding reduction of dextrose-corn starch content. Animals on this low calorie diet were fed half the dry weight per day actually consumed by low protein diet animals, thus receiving similar intakes of all components except carbohydrate. A fourth group of animals was fed the 6% casein diet *ad libitum*, and in addition received two intraperitoneal injections of 0.1 ml. killed pertussis vaccine (approximately 10⁹ organisms) 1 week apart. All groups of mice were commenced on diets at 21 days of age and continued for 14 days, following which all groups received the full 28% casein diet for the duration of the experiment.

C₃H₇/Umc mice in the four early dietary groups were inoculated with 30 × 10⁶ viable DBA/2 P-815-X2 mastocytoma ascites tumour cells by intraperitoneal injection at 10 weeks, 13 weeks and 17 weeks of age (after 5 weeks, 8 weeks and 12 weeks on the full protein diet). Spleens, lymph nodes and sera were collected 11 days later. Cytotoxic cellular immunity was measured in this allogenic system by the mastocytoma ⁵¹Cr release method of Brunner⁶ as modified by Canty and Wunderlich⁷. Percentage specific lysis was calculated from the formula:

Percentage specific lysis =

$$\frac{\text{c.p.m. of specimen} - \text{c.p.m. with non-immune cells} \times 100}{\text{total releasable activity} - \text{c.p.m. with non-immune cells}}$$

Radioactivity was measured using a well-type gamma counter (Nuclear Chicago Corp.). All experiments were performed in triplicate.

Haemagglutinating antibody was determined by the PVP method⁸ using allogenic erythrocytes from DBA/2 mice. Results were expressed as mean reciprocal log titres.

The cytotoxicity *in vitro* of lymphoid cells by anti-theta-C₃H antibody and rabbit complement was performed by a two-stage test described by Raff and Wortis⁹. Anti-theta-C₃H serum

Table 1 Long Term Effects of Nutritional Deprivation at Weaning on Cytotoxic Cellular Immune Responses Tested by *in vitro* Assay after 5, 8 and 12 Weeks on a Normal Diet

Diets at weaning	Total calories/mouse day	5 weeks		Cell-mediated lysis 8 weeks		12 weeks	
		C.p.m. ± s.d. *	% Lysis †	C.p.m. ± s.d.	% Lysis	C.p.m. ± s.d.	% Lysis
28% Casein	8.5	4,178 ± 396	82	4,036 ± 421	78	4,294 ± 398	84
6% Casein	8.0	1,452 ± 189	18‡	2,058 ± 245	32‡	4,028 ± 476	78
6% Casein half calorie	4.0	707 ± 131	8‡	1,322 ± 141	15‡	3,057 ± 321	56‡
6% Casein pertussis	9.0	4,170 ± 449	81	3,962 ± 381	76	3,763 ± 411	72
Non-immune mice		674	0	668	0	584	0

* Mean c.p.m. and s.d. ⁵¹Cr released from DBA/2 mastocytoma target cells during 2 h incubation with sensitized C₃H spleen cells at 1:100 ratio. Total releasable activity in each test was adjusted to 5,000 c.p.m. There were ten mice per group.

† Percentage specific lysis of target cells.

‡ Significantly different from values in normal diet mice ($P < 0.01$).

was absorbed *in vitro* with AKR thymocytes¹⁰ and *in vivo* in theta-deprived CBA/H mice¹¹⁻¹².

The mean body weights of mice fed both the low-calorie diets (64% of mean body weight of controls) and low-protein diets with antigenic stimulation (67% of controls) during the 2 weeks after weaning, remained significantly lower than control mice fed the high-protein diet when measured 8 weeks after ceasing the restricted diet.

Cytotoxic cell mediated immunity, measured by *in vitro* assay (Table 1), remained normal in animals fed 28% casein diets and also in those fed low-protein diets with antigenic stimulation. Animals fed low-protein diets at weaning showed decreased cytotoxic cellular immunity 5 weeks and 8 weeks after returning to a normal diet, but by 12 weeks these responses had returned to normal. Animals fed the more severe low-protein-low-calorie diet after weaning showed a more severe deficit of cytotoxic cell mediated immunity after 5 and 8 weeks on a normal diet and recovery was incomplete at 12 weeks.

Table 2 Long Term Effects of Nutritional Deprivation at Weaning on Haemagglutinating Antibody Titres tested 12 Days after Primary Allogenic Tumour Cell Inoculation and after 5, 8 and 12 Weeks on a Normal Diet

Diets at weaning	Mean log haemagglutination titre	5 weeks	8 weeks	12 weeks
28% Casein		4.3	4.5	4.4
6% Casein		3.6*	4.1	4.2
6% Casein half calorie		2.7*	3.9	3.9
6% Casein pertussis		4.8	4.9	4.7

* Significantly different from normally fed mice ($P < 0.01$).

Table 3 Long Term Effects of Nutritional Depletion at Weaning on Lymphoid Cell Population in Spleen Lymph Nodes and Thymus

Diets at weaning	Weeks on normal diet	Total No. lymphoid cells spleen*	Percentage theta positive †	Spleen	Lymph nodes	Thymus
28% Casein	5	128 ± 8.2		36	52	79
	12	136 ± 7.3		34	48	76
6% Casein	5	92 ± 5.2		16 ‡	32 ‡	57 ‡
	12	114 ± 6.5		32	36 ‡	77
6% Casein half calorie	5	76 ± 4.1		11 ‡	21 ‡	49 ‡
	12	93 ± 4.8		26 ‡	34 ‡	72
6% Casein pertussis	5	145 ± 8.3		33	46	77
	12	163 ± 8.7		29	49	79

* Mean ± s.d. × 10⁶.

† Calculated from the number of mononuclear cells per organ, the percentage lymphocytes in stained smears and the percentage reduction in mononuclear cells after treatment with anti-theta serum compared to reductions after normal AKR serum.

‡ Significantly different from normal animals ($P < 0.01$).

Decreases in haemagglutinating antibody responses were significant only in the low-protein and low-protein-low-calorie groups of mice when tested after five weeks on a normal diet (Table 2).

After 5 weeks on the normal diet both low-protein and low-protein-low-calorie groups of animals had significantly reduced numbers of theta-positive lymphoid cells in spleen, lymph nodes and thymus. By 12 weeks on the normal diet both groups had more normal proportions of theta-positive cells in spleens (Table 3).

Severe protein calorie malnutrition causes extensive changes in the structure and function of the immune system in man and animals^{13,14}.

In this study, mice subjected to nutritional stress at the time of weaning showed persistent deficits in cytotoxic cellular immune function, reduced numbers of thymus derived (theta-positive) lymphoid cells in spleen, thymus and lymph nodes and diminished specific antibody responses.

The depression in cell mediated immunity and relative deficits of theta-positive cells in spleens and lymph nodes, were proportional to the degree of early nutritional deprivation both in extent and duration before recovery. These findings show a striking parallel with the effects of surgical removal of the thymus at a similar age¹⁵ and suggest a maturation arrest of the thymus-dependent lymphoid system through "nutritional thymectomy" in infancy. Other factors, such as the presence of an immuno-suppressive mouse virus activated by nutritional stress cannot, however, be excluded.

Animals in this study who received intense antigenic stimulation, in addition to a restricted protein diet, showed no demonstrable deficit in immune function when tested 5 weeks later. Their mean body weights were, however, significantly lower than mice fed the low-protein diet alone.

Severe kwashiorkor commencing very early in infancy has been associated in man with profound depression of total serum immunoglobulin levels and immunological defects, components of which persisted after refeeding¹⁶, suggesting a maturation arrest of the B cell system similar to the arrest in the T cell system suggested in this study, and by our previous observations in man⁵.

We thank Mrs C. Blaska for technical assistance. This study was supported by grants from the National Foundation—March of Dimes, the American Heart Association, US Public Health Service. D. G. J. was supported by a C. J. Martin fellowship of the National Health and Medical Research Council of Australia.

D. G. JOSE*
O. STUTMAN
R. A. GOOD

Research Laboratories of the
Variety Club Heart Hospital,
Departments of Immunobiology and Pathology,
University of Minnesota,
Minneapolis,
Minnesota 55455

Received August 7; revised November 1, 1972.

* Present address: Royal Children's Hospital Research Foundation, Melbourne, Australia 3052.

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Seasonal Variations in Bone Mineral Content after the Menopause

PHOTON absorption techniques for measuring bone mineral content *in vivo*¹ have made possible realistic assessment of short-term sequential changes in skeletal mass in human subjects. Shimmins *et al.*², using a photon absorption technique to measure the mineral content of the third metacarpal of human volunteers claimed a reproducibility for the method of $\pm 3\%$ (1 s.d.). In post-menopausal women there is skeletal wasting resulting in an average fall in bone mineral content of about 1% per year³ (post-menopausal osteoporosis⁴). Successive measurements of bone mineral content made in a group of post-menopausal women at six-monthly intervals would be expected to show a mean fall of only 0.5% on each occasion. The difference between any two such measurements should be within the reproducibility of the method, $\pm 3\%$. Preliminary investigations using this method for confirming the expected reproducibility of the method gave results very much greater than $\pm 3\%$, inferring that either gross technical irregularities had appeared or specific biological changes were occurring. A more detailed investigation of the problem revealed some unexpected and interesting results.

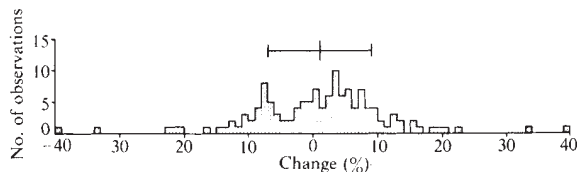


Fig. 1 Histogram showing percentage six-monthly change in metacarpal mineral content in 55 post-menopausal women.

Fifty-five post-menopausal women attending a clinic because of skeletal pain were examined. We measured metacarpal mineral content at six monthly intervals for up to two years, using a photon absorption technique². The percentage difference between successive measurements was calculated, and the 130 results were plotted as a histogram (Fig. 1). The mean percentage error of $\pm 8\%$ was more than twice the expected error, but the distribution suggested the existence of two separate populations; one recording predominantly negative changes and the other predominantly positive changes, with the maxima at -8% and $+4\%$ respectively. Because these women were somewhat heterogeneous, and many were receiving a variety of different medicaments, we extended the investigation to a group of healthy post-menopausal women.

that there was an overall rise from January to July with the former being 3.9% lower and the latter 4.8% higher than the previous measurement ($t=2.52$, $P<0.05$). Similarly the differences between May and November were $+2.7\%$ and -2.9% respectively ($t=2.53$, $P<0.02$), and the differences between June and December were $+4.4\%$ and -4.7% respectively ($t=2.65$, $P<0.02$). Only very small differences were seen when consecutive measurements were made in March and September and April and October and vice versa. Since the number of observations in each month was small, the mean change in the whole of the winter period (November to February) was compared with the mean change for the whole of the summer period (May to August). The mean winter change ($\pm$ s.e.m.) was -4.01% (± 1.21), whereas the mean summer change was $+3.63\%$ (± 1.10). These differences were very highly significant ($t=4.67$, $P<0.00001$). The mean differences from March and April to September and October remained insignificant.

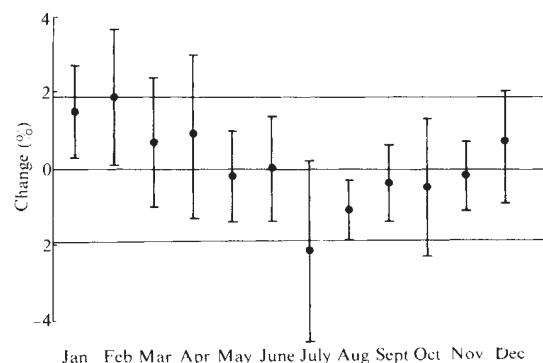


Fig. 2 Percentage change in aluminium standard according to month in which measurement performed. (0% = overall mean; means $\pm$ 1 s.d. shown for both overall mean and monthly means.)

To ensure that these trends were not methodological in origin an aluminium standard, which introduced attenuation of the photon beam comparable with a human metacarpal, was scanned under the same conditions at frequent intervals throughout the second year of the study. During this period 78 standard scans were made of which 95% were within $\pm 3.8\%$ of the mean. The extent to which the mean of all the standard scans done in any one month deviated from the overall mean is shown in Fig. 2. The mean percentage difference found for each of the twelve months did not differ appreciably from the overall mean. Furthermore, the lowest mean standard

Table 1 Percentage Six-monthly Change in Metacarpal Mineral Content in 48 Post-menopausal Women, according to the Month in which the Last Measurement was Made

Month	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
No. of observations	8	10	16	16	11	13	8	10	16	16	11	13
Mean percentage change ($\pm$ s.e.m.)	*-3.9 (± 3.0)	-4.4 (± 2.6)	-0.9 (± 1.6)	-1.2 (± 1.6)	†+2.7 (± 0.8)	†+4.4 (± 2.5)	*+4.8 (± 1.7)	+2.7 (± 3.1)	+1.3 (± 2.6)	-0.7 (± 1.1)	†-2.9 (± 2.1)	†-4.7 (± 2.4)

* $P<0.05$. † $P<0.02$.

Forty-eight healthy female volunteers aged 36–56 yr who had at some time in the previous six years undergone bilateral ovariectomy and who were thereby post-menopausal had repeated measurements of metacarpal mineral content made at six-monthly intervals for either one or two years. The percentage differences between consecutive six-monthly measurements were grouped according to the month of the year in which the latest measurement was performed (Table 1). It can be seen

reading was found in July, when the *in vivo* measurements were highest and the highest mean standard readings were found in January and February when the *in vivo* measurements showed some of the greatest falls. Hence the small trends which were found in the standard scans would have tended to nullify the *in vivo* changes which were actually observed and which might therefore have been even more significant, had the variations found in the standard scans not been present.

This investigation has shown that highly significant seasonal variations in bone mineral content occur, with increments from winter to summer and decrements from summer to winter; little change being seen from spring to autumn or vice versa. The increments could be explained by an increase in vitamin D activity from increased exposure to sunlight in the spring and summer. This would infer the presence of asymptomatic vitamin D deficiency. The total surface area of osteoid in the bones increases with age in women after the fourth decade of life⁵, suggesting defective mineralization of osteoid as is seen in vitamin D deficiency states. Serum vitamin D activities in post-menopausal (Michigan) women are higher in the summer than the winter⁶. Also serum phosphorus⁷ and calcium concentrations rise from winter to summer, and this is consistent with a humoral response to vitamin D. Whether or not such humoral changes could explain our findings must await further investigation, but the possibility that sub-clinical vitamin D deficiency might be a contributory factor in post-menopausal osteoporosis is of considerable interest.

This work was supported by grants from the Scottish Hospital Endowments Research Trust, the National Fund for Research into Crippling Diseases and G. D. Searle and Co., Ltd, High Wycombe.

J. M. AITKEN
J. B. ANDERSON

*Bone Metabolism Research Unit,
University Department of Medicine,
Western Infirmary,
Glasgow W1*

P. W. HORTON

*Regional Department of Clinical Physics
and Bio-engineering,
Western Regional Hospital Board,
11 West Graham Street,
Glasgow C4*

Received April 27; revised July 27, 1972.

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Tissue Zinc in Malignant Disease

It was reported by two of us that liver-zinc concentration is significantly higher in subjects dying from malignant disease than in non-malignant control series¹. The increase was confined to parts of the liver which showed no macro or microscopic evidence of carcinomatous invasion. (The zinc content of the malignant deposits themselves is lower than that of normal liver.) Three explanations were considered. First, it was possible that the rise in liver zinc reflected a widespread premalignant change not peculiar to the liver. Second, it could be related to the poor nutritional state of most patients dying from malignant disease. Third, it could be a feature of the chemical defence reaction of normal liver tissue to invasion by malignant cells. These possibilities were further explored by measuring the zinc concentration in the liver, kidneys, heart muscle, spleen and pancreas of a further series of subjects. The analytical methods were as reported previously^{1,2}. The results (Table 1) confirm the increase in liver zinc in apparently normal tissue in subjects dying from carcinoma but show no

comparable increase in the zinc concentration of the kidney, heart, spleen and pancreas. The scatter of results in lung tissue was too wide to allow a firm negative conclusion.

Table 1 Tissue Zinc Levels in Subjects with and without Malignant Disease

Tissue	Malignant disease		Control series		P
	Mean	s.d.	Mean	s.d.	
Liver	837	204	538	95	<0.05
Kidney	502	154	505	106	>0.49
Heart	301	98	364	69	>0.25
Spleen	169	64	196	42	>0.25
Pancreas	263	104	291	126	>0.30

Zinc values in mg per 100 g ashed tissue.

The findings make it unlikely that the accumulation of zinc in the apparently healthy liver of carcinomatous subjects is a reflexion of a widespread premalignant change; and it is similarly improbable that the liver alone would be affected by the patients' poor nutritional state. We therefore conclude that liver zinc concentration rises probably as part of the normal tissue's biochemical defence reaction to invasion by malignant cells. Such an invasion in the course of a fatal malignant disease is probably invariable, the vast majority of invading cells being eliminated by the host tissue. Zinc also accumulates in granulation tissue and in and around healing wounds^{3,4}; and it is conceivable that the two reactions have biochemical features in common.

K. GRIFFITH
E. B. WRIGHT
T. L. DORMANDY

*Department of Chemical Pathology,
Whittington Hospital,
London N19*

Received August 7, 1972.

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Cannabis Induced Impairment of Performance of a Divided Attention Task

AN important factor in driving skill is the information processing ability of the driver. The human operator can process less information within a certain time if it must be received from more than one source¹. Driving is such a situation and can be described as a divided attention task in which the driver is forced to perform a compensatory tracking task while searching for and recognizing environmental signals². Our subjects were required to monitor and respond to two types of visual signals from different sources. We compared the effect of cannabis on the performance of subjects with and without previous experience of cannabis.

Ten male volunteer subjects had not used marihuana before the experiment and ten were experienced social users. Median use was once a week for three years but this varied within the group. The two groups were matched for age and education.

Cigarettes were prepared containing 700 mg of placebo material (extracted plant material, smelling and tasting very similar to the active material), 450 mg placebo and 250 mg active material, and 200 mg placebo and 500 mg active material. The cannabis, assayed by gas chromatography, was found to contain 1.32% Δ^9 -THC giving an administered dose of 3.3 mg of Δ^9 -THC in the low dose cigarettes and 6.6 mg at the higher dose level (Casswell and Marks, in preparation).

Using a double blind design the placebo and two dose levels of cannabis were administered in counterbalanced order on three separate testing occasions, approximately one week apart. A standard paced method of smoking was used in which each inhalation was held for 30 s. The naive subjects were all tobacco smokers and able to inhale. A highly significant ($P < 0.001$) dose-related effect of cannabis on pulse rate indicated that Δ^9 -THC was being absorbed by both naive and experienced subjects. The divided attention task was started 30-40 min after the subjects had finished smoking the cigarette.

The subject faced a central light surrounded by 10 peripheral lights spaced at 15° intervals on a 150° horizontal perimeter. The central light flashed at the rate of one per second with randomly programmed breaks in the flashes at the rate of one break every 20 s. The subject was required to press a key every time he detected a break in the sequence of centre light flashes. The peripheral lights were programmed to flash randomly with each light flashing 12 times during the 40 min the subject performed the task. The subject was required to press a second key in response to a peripheral light signal.

Significantly more of both central light signals and peripheral light signals were missed by the subjects after smoking cigarettes containing Δ^9 -THC than after smoking placebo material. Another result of major interest was the lack of significant difference between experienced and naive subjects in the amount of impairment obtained.

Table 1 Mean Number of Signals Missed following the Three Administrations for Naive and Experienced Subjects

Δ^9 -THC (mg)	Mean No. of misses centre light signal		Mean No. of misses peripheral light signal	
	Naive	Experienced	Naive	Experienced
0	1.6	2.4	6.1	5.0
3.3	4.3	6.2	9.0	9.1
6.6	8.1	7.4	13.7	9.3
F values	9.25		4.19	
P values	<0.001		<0.01	

The results were tested using two-way analyses of variance. F values are for drug-level effect. Between groups effects were non-significant for both central and peripheral signals.

This decrement in performance on a divided attention task is similar to that found following alcohol intoxication which is believed to be associated with the frequent occurrence of alcohol-related accidents³.

These results, showing impairment on a dividend attention task following doses of Δ^9 -THC comparable to those used socially⁴, support a previous finding of impairment of performance of a combined tracking and stimulus recognition task following an unknown amount of cannabis smoked in a social setting⁵. Results showing approximately equal impairment for experienced and naive subjects conflict with reports of experienced users that they do not have trouble driving when they are "high"⁷. Further, an investigation of cannabis effects on performance in a driving simulator reported no decrement in performance on several variables which have been shown to be correlated with driving performance⁷. The discrepancy between the present results and both previous laboratory results and users' reports might be explained by a difference in motivation of the subjects. The simulator task is shorter and probably more intrinsically interesting than the divided

attention task used in this study. People driving after use of marihuana will of course have the threat of personal injury and expense to motivate their performance. Grinspoon⁸ has suggested that motivation is an important variable in the performance of experienced users of marihuana. Motivation of subjects in a laboratory situation during cannabis intoxication must be investigated before results such as ours can be interpreted in relation to driving ability. An investigation of the motivation variable in relation to cannabis effects on performance is under way.

We thank Professor R. W. Medlicott for screening the subjects and Mr D. Ferry and his co-workers in the MRC Toxicology Unit, Otago University, for preparation and analysis of the material. This research was financed by a grant to Professor P. McKellar from the Medical Research Council of New Zealand.

SALLY CASSWELL
D. MARKS

Department of Psychology,
University of Otago,
PO Box 56,
Dunedin

Received July 21; revised September 7, 1972.

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A New Constituent of Biogenetic, Pharmacological and Historical Interest from *Melaleuca cajuputi* Oil

CAJUPUTI oil, obtained by the steam distillation of leaves of *Melaleuca cajuputi* Powell (Myrtaceae), has had a wide range of medical applications in the Indo-Malaysian region for many years¹. This reputation caused it to be one of the first products brought by the Dutch to Europe from SE Asia. Several early eighteenth century publications² related to its introduction into Europe by the Dutch, but present day requirements are met by leaf oils of related *Eucalyptus* species. There have been, however, consistent reports regarding antiseptic and analgesic properties of cajuputi oil, which are hardly accounted for by the reported composition. This is largely cineole, together with pinene, terpineol and its simple aliphatic esters, and traces of benzaldehyde and valeraldehyde².

Silicic acid chromatography of a sample of the oil obtained from *M. cajuputi* trees on the campus of the University of Malaya revealed the presence of substantial quantities (about 10%) of a previously unrecorded crystalline phenolic constituent. This had the formula $C_{12}H_{16}O_4$, contained a methyl ketone system, and had mass, n.m.r., ultraviolet and infrared spectra consistent with the compound being 3,5-dimethyl-4,6-di-O-methylphloroacetophenone. This was confirmed by demethylation to 3,5-dimethylphloroacetophenone³.

The former compound is a new natural product, and its occurrence is of interest for several reasons. First, in biogenetic terms, it is the most highly methylated simple acetogenin known, with C- or O-methylation at all probable positions. The remaining free phenolic group is strongly hydrogen-

bonded, and in analogous situations (for example, the 5-hydroxyl of flavones) is rarely substituted. This emphasizes a tendency for production of highly alkylated acetate-derived polyphenols in the Myrtaceae, other examples being the chromones of *Eugenia* and *Backhousia*, phloroglucinols in *Baeckea*, and flavones in *Eucalyptus*⁴.

Second, as simple phenols have antibiotic properties and these are increased markedly by alkylation of the aromatic ring⁵, the occurrence of substantial quantities of this compound in cajeputi oil provides a rational explanation for the anti-septic properties. More speculatively, oxidative degradation of the compound under crude distillation conditions might well yield (as the aromatic ring is protected by complete substitution) a salicylic acid homologue with analgesic properties.

The third point is in relation to the past trade in cajeputi oil. Early European supplies were coloured green, and partly from confusion regarding the sources and production of the oil, this came to be regarded as an essential attribute. A suitable dye was often added to those batches that did not possess the "correct" colour, and eventually the green colour was linked with the use, in some localities, of copper distillation vessels for production of the oil. This is hardly a complete explanation, however, as the recorded constituents should have no interaction with copper, and the effect has been attributed to such causes as traces of butyric acid released from the terpinyl ester, itself a minor constituent. The β -hydroxyketo grouping present in 3,5-dimethyl-4,6-di-O-methylphloroacetophenone is an extremely effective chelating system for metals, and the ease with which it forms a soluble complex with copper provides the other half of the answer to an early mystery of the spice trade.

J. B. LOWRY

Department of Chemistry,
University of Malaya,
Kuala Lumpur

Received September 26, 1972.

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Evidence for Apostatic Selection by Predators using Olfactory Cues

MANY visual predators hunt in a frequency-dependent manner, and can actively maintain colour polymorphisms in their prey¹⁻³. These predators tend to concentrate on common varieties of prey, and to overlook rarer forms even if they are obvious. This type of behaviour is responsible for apostatic selection³, and has been interpreted as being due to the formation of "specific searching images"⁴⁻⁶.

We have tried to discover whether predators behave in a similar manner when they are using olfactory, rather than visual, cues. As predators we used an outbred laboratory stock (Edinburgh Q) of *Mus musculus* L. The "prey" were artificial baits, cylindrical in shape (6 mm in length, 3 mm in diameter) made of five parts by weight plain flour and four parts lard. They were scented with peppermint essence (J. M. Bannerman and Co. Edinburgh: 2.5 ml. to 40 g dough) or vanilla (Crosse and Blackwell Ltd London: 5 ml. to 40 g). Edible brown dye (Scottish Flavour and Colour Co. Ltd Edinburgh) was added

to give them identical value ("lightness") and chroma (strength of colour) and nearly-identical hue on the Munsell Scale⁷. The colour-scores were 2.5 Y 4/8 for peppermint-flavoured baits and 2.5 YR 4/8 for vanilla. A slight difference in hue, just visible to the human eye, was due to colouring already present in the vanilla essence.

The predators were conditioned by exposing them to twenty baits of one variety for a period of ten minutes on three consecutive days. Variation between individuals was reduced by hand-feeding each mouse when necessary to ensure that it had eaten a total of twenty baits during the conditioning period. Tests were carried out on the fourth day, offering the predators equal numbers of the two types (ten of each) set out in an alternating rectangular array. Conditioning and testing were conducted in the same area against a background of stiff white paper (2.5 R9N⁷) replaced after each test, and under standard illumination (an 80 W fluorescent lamp situated five feet vertically above the area). The test area was 17" × 14". The analysis was based on the first ten baits attacked by each individual during the test period. All attacks (contacts between the bait and the mouth of the predator) were counted, including those in which the baits were not eaten. To the human eye the peppermint baits (P) seemed to be more conspicuous. The vanilla baits (V) appeared to be more palatable to the mice. The probabilities quoted in the text are derived from contingency tables using the numbers of mice showing particular preferences, not the numbers of baits attacked. This avoids the possible pitfalls of assuming that each attack is an independent event.

In the first experiment, thirty naïve adult female mice were allowed their normal diet of rat cake (Edinburgh University Diet) while being trained and tested on the different odours. The results (Table 1a) show that mice trained on P baits attacked significantly more P than V ($P < 0.02$) and that those trained on V attacked significantly more V than P ($P < 0.001$). The difference in the behaviour of the two groups is highly significant ($P < 0.001$).

Because the response of a predator to a particular prey can be altered by changes in the relative palatability of alternative food⁸, the first experiment was repeated, under identical conditions except that rat cake was replaced by sunflower seeds, which are taken more readily by *Mus*. This might be expected to reduce the effectiveness of conditioning. Although the conditioning did indeed appear to be less effective (Table 1b), the results of the second experiment are not significantly different from those of the first.

Six mice that had been trained on P and six that had been trained on V were retested five days later, having meanwhile been fed on rat cake. The results (Table 1c) show that each group maintained its preference even after contact with the alternative bait. The difference between the two groups remains highly significant ($P < 0.005$).

Eighteen mice from the original groups were retrained for three days on alternative baits to those on which they had been trained. The results given in Table 1d suggest that retraining has destroyed the earlier preferences. Neither group shows a significant departure from random predation, nor is there any difference between them.

Among visual predators, there is evidence that when a particular prey is at high density and there is a lack of alternative prey, "satiety" may develop⁵, manifesting itself as a decreased tendency to take prey that has previously been common. An experiment was carried out to test whether this effect occurs among olfactory predators. Eighteen mice were divided into two groups, each of which was offered an excess of one type of bait for three days without alternative food. They were then tested in the same manner as before. The results (Table 1e) show that each group developed a preference for the unfamiliar baits ($P < 0.001$). Trials with further groups of mice showed that a single day of training with excess P induced a preference for V, although the preference became more pronounced with further training. On the other hand,

Table 1 Predation by Mice of Artificial Baits Scented with Peppermint (P) or Vanilla (V)

	P trained	Total	Previous total by same mice	V trained	Total	Previous total by same mice
	Baits attacked by individual mice			Baits attacked by individual mice		
a	P 2 7 7 6 5 7 5 6 7 5 6 6 7 5 7 V 3 3 3 4 5 3 5 4 3 5 4 4 3 5 3	88 57	— —	1 2 4 2 2 4 3 3 4 5 0 0 2 2 1 4 8 6 5 8 6 7 7 6 5 4 6 8 8 9	35 97	— —
b	P 1 4 5 5 5 4 V 0 6 5 5 5 6	24 27	— —	3 4 3 3 1 5 7 6 7 7 9 5	19 41	— —
c	P 7 5 6 5 7 7 V 3 5 3 5 3 3	37 22	37 23	1 1 1 0 4 2 9 9 6 9 6 8	9 47	13 44
d	P 6 4 4 4 4 5 5 5 3 V 4 6 6 6 3 5 5 5 5	40 45	20 62	4 4 6 5 5 4 5 5 6 6 6 4 5 5 6 5 5 3	44 45	54 39
e	P 1 0 0 3 0 1 1 0 1 V 9 2 3 7 6 8 2 1 9	7 47	— —	7 4 2 0 6 2 0 1 2 3 3 1 0 0 1 2 1 1	24 12	— —

a, One set of mice had been conditioned ('trained') by exposure to P baits, the other set by exposure to V baits. The alternative food was rat cake. b, Experiment identical to (a) except that the alternative food was sunflower seeds. c, Mice from experiment (a) were retested after an interval of five days. d, Results of 'retraining'. Mice from experiment (a) that have been trained on V were retrained on P, and vice-versa. e, Training by exposure to an excess of one type of prey. Note reversal of preferences compared to experiment (a).

a single day of training with excess V induced a preference for V, two days' training led to no preference, and three or more days' training induced a preference for P. Thus it seems that the same bait in different circumstances can be treated as palatable or unpalatable (cf. Young^{9,10}).

We have found some evidence that mice can discriminate visually by tone (value⁷) rather than colour (hue⁷). Experiments using baits made distasteful with quinine suggested that the mice could discriminate between baits differing by 5 units of value on the Munsell Scale¹¹. It therefore might be argued that the results of our conditioning experiments were due to visual rather than olfactory factors. This argument can be discounted, for several reasons: (i) The P and V baits differed only very slightly in hue, and not at all in value. There is no clear evidence of colour discrimination in mice¹². (ii) Experiments in which mice were conditioned to unflavoured baits differing in value (5 units on the Munsell Scale) gave no evidence that visual preferences were developed¹¹. (iii) We trained one set of three mice on dark V, and another on light P. The dark and light baits differed by five units of value. The mice were then tested on dark P and light V. The results (Table 2) show that olfactory preferences dominate any possible visual ones. The differences are significant ($P < 0.05$, one-tailed) and entirely compatible with the earlier experiments.

A small additional series of experiments, similar to those already described but using long-tailed field mice (*Apodemus sylvaticus* (L.)) captured from wild populations, suggested that these predators can also develop olfactory preferences¹¹. There is evidence that deer-mice (*Peromyscus* spp.) behave in a similar manner¹³.

We conclude that predators hunting by smell can develop preferences for familiar types of prey. Among visual predators this kind of behaviour is capable of maintaining colour polymorphisms within the prey species¹⁻³. It follows that there should be scent polymorphisms within species attacked by olfactory predators. To our knowledge, however, the only

known cases of animal scent polymorphisms are Müller's¹⁴ report of dimorphism in male butterflies (*Battus polydamus*) and some associations with mimicry¹⁵. Their apparent rarity may be due only to the difficulty of detecting them. Our experiments lead us to believe that this rarity is indeed more apparent than real. They also suggest that it would be profitable to search for the development of preferences among predators using other senses (the use of auditory cues by nocturnal predators, or pressure and electric fields by aquatic predators). We would expect to find corresponding polymorphisms (in sound, movement or electrical properties) among their prey.

We thank Mrs A. G. Clarke, Mrs G. A. Soane, Dr T. H. Day and Dr D. T. Parkin for critically reading the manuscript, and the Science Research Council for financial support. The work was carried out at the Department of Zoology, University of Edinburgh, and we thank Professors J. M. Mitchison and P. M. B. Walker for the help and facilities they have provided.

I. D. SOANE

School of Plant Biology,
University College of North Wales,
Bangor,
Caernarvonshire

B. CLARKE

Department of Genetics,
School of Biological Sciences,
University of Nottingham,
University Park,
Nottingham NG7 2RD

Received July 21, 1972.

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Table 2 Tests for Visual Preferences

i, Trained on 'light' P	Total	ii, Trained on 'dark' V	Total
No. of dark P attacked	6 2 6 14	3 2 3	8
No. of light V attacked	4 2 4 10	7 8 7	22

Mice were trained on 'light' P and 'dark' V, but tested on 'dark' P and 'light' V. 'Dark' and 'light' baits differed by 5 units of value on the Munsell Scale⁷.

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Control of Aggression by Singing in Crickets

ETHOLOGISTS developed the idea that many animal species evolved mechanisms to control aggression among their members. This control is by the use of signals to reduce the probability of attacks by the opponent before injurious or lethal combats occur¹⁻³. Although there are observations that are best explained by the theory, no direct experimental evidence has been presented. We report here one type of experimental evidence that seems to fit the theory.

Cricket fights have been a popular sport in China since ancient times⁴. When two adult male crickets are put together in an empty aquarium containing just one burrow for hiding, they fight over it. The winner and the loser are determined during the initial phase through vigorous battles. After this period, actual physical combats occur less frequently because the subordinate cricket tends to avoid encounters with the dominant one either by not approaching it or by getting away from it.

Acoustic signals seem to play an important role in the proceeding and outcome of an encounter. Adult male crickets produce several types of sound. A male, perched alone at the entrance of his burrow, gives the calling song, the most commonly known chirping of crickets. When two male crickets come close or engage in a battle, they raise their wings and produce the so called aggressive or rivalry song. Dominant males sing more aggressive songs than subordinate males and the amount of aggressive stridulation is positively correlated with winning encounters⁵.

We have found that deafening an adult male cricket immediately makes it more aggressive. Crickets of the species *Acheta domesticus* and *Gryllus pennsylvanicus* were deafened by tearing the tympana in the forelegs under ether anaesthesia. This operation abolished the responses of the tympanic nerve to a play-back of recorded chirps as monitored electrophysiologically. The sub-genual organ in the foreleg and the cerci responded to low frequency tones. These organs perhaps cannot register the stridulatory sounds, the frequency range of which lies between 3 kHz and 6 kHz in *A. domesticus* and 3.5 kHz and 5 kHz in *G. pennsylvanicus*. The subgenual organs in the middle pair of legs responded to recorded chirps played back at supernormal intensity. Control experiments showed that ether had no effect on the aggressiveness of the cricket.

Individuals were marked with various combinations of dots of silver enamel. In the first series of experiments, two unacquainted crickets were put together in a small cage which made frequent encounters inevitable. In this situation the crickets fought or demonstrated the dominance relationship clearly. We observed each pair of crickets until a statistically sufficient number of encounters was recorded. As Alexander⁵ noted, the results of most encounters between male crickets are clear cut. In this report, we include only those encounters in which one cricket dominated the other as evidenced by the retreat or evasive behaviour of the subordinate individual after physical combats. The loser was then deafened and rematched with the winner. One example of *G. pennsylvanicus* is presented (Table 1). The deafened cricket which had lost the majority of encounters now clearly dominated its former superior rival. In all cases, the deafened losers immediately became extremely aggressive; they initiated combats and engaged in sustained battles.

In the second series of experiments three or four crickets were kept together in an empty aquarium where they freely

Table 1 Effects of Deafening on Fighting

	<i>G. pennsylvanicus</i>		Fisher exact probability test
Crickets	Before	After	
1→2	30	4	$P < 0.00001$
2*→1	2	25	
	<i>A. domesticus</i>		
	Before	After	
2→4	10	2	$P = 0.00008$
4*→2	2	11	
3→4	13	2	$P = 0.00023$
4*→3	1	6	
2→3	2	2	$P = 0.371$
3→2	17	11	

Figures under "before" and "after" indicate the numbers of winning encounters before and after deafening. $x \rightarrow y$ indicates x won over y . The Fisher exact probability test was used to obtain the significance level of the differences in the number of winning encounters between the matches before and after deafening. Before deafening *A. domesticus* had established a dominance hierarchy in which 3 dominates 2 dominates 4. This was reversed after the deafening of cricket 4.

* Deafened cricket.

encountered and could establish a dominance hierarchy. Again two crickets were transferred at a time to the small test cage for a bout of encounters. Each insect met every other in a tournament. This procedure enabled us to determine quickly the rank of each insect in the hierarchy. The lowest ranking insect was then deafened and rematched with all the higher ranking individuals. One example of *A. domesticus* is presented in Table 1.

This group contained four insects numbered 1, 2, 3 and 4, which had established the following dominance hierarchy $3 > 1 > 2 > 4$, where $x > y$ indicates x dominates y . After the deafening of the lowest ranking cricket 4, a new rank order $4 > 3 > (1) > 2$ resulted. Since cricket 1 died before the completion of the tests, its rank was uncertain. The deafened cricket 4 dominated significantly larger numbers of encounters in the rematches with crickets 2 and 3. In the contest between the intact crickets 2 and 3, no significant change occurred between the initial match and the rematch.

In another group of four *A. domesticus* the deafened lowest ranking cricket climbed but one place in the hierarchical ladder. It could not dominate the remaining two rivals. The results of the tournament show, however, that in every rematch, the deaf cricket significantly increased the proportion of winning encounters. Alexander⁵ reports that the dominance-subordinate relationship between two males could be reversed by forcing the dominant cricket to lose fights against a dummy while broadcasting synthetic or natural rivalry songs. This observation not only lacks statistical validation but also does not isolate the role of the acoustic signal, since the abortive physical engagement (antennal lashing) with the dummy alone may account for the reversal of the rank order.

We offer the following hypothesis to explain our results. The acoustic signals of the dominant cricket inhibit the aggressive tendency of the subordinate one. The inability to hear the signals of the opponent frees the deaf cricket from the inhibition. The consequence is less inhibited aggression. The incomplete reversal of rank mentioned above seems to indicate the existence of some other factors which influence the probability to win an encounter. The calling song of a male cricket not only attracts females⁶ but also perhaps "warns" other males at a distance. The rivalry song which is used at a close range perhaps plays a more important role in the control of aggression.

This work was supported by a grant from the National Science Foundation.

L. H. PHILLIPS II
M. KONISHI

Department of Biology, Princeton University,
Princeton, New Jersey 08540

Received September 18, 1972.

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Population Control in Snails by Natural Inhibitors

IN many areas of the world diseases such as schistosomiasis caused by trematodes, still cause misery to millions of people and much damage to livestock. Because a specific mollusc, often a snail, is necessary for the completion of the trematode life cycle, the elimination of that host would eradicate the disease. According to Southgate¹ none of the molluscicides currently available is ideal. Among their disadvantages are toxicity to organisms such as fish and the relatively high concentrations required for their effect. Here we describe a naturally occurring population inhibitor from snail cultures which because of its specificity and potency suggests another approach to mollusc control. The data presented below indicate that such substance is produced in cultures of at least two species of snails. Discovery of this inhibitor came about through an investigation into the chemical and physiological basis for the inhibition of population growth which occurs in crowded snail cultures.

Rose² reviewed the literature describing population restriction by crowding and indicated that it is common in plants and animals. Among molluscs, Chernin and Michelson^{3,4} reported that a snail host of schistosomiasis, *Biomphalaria glabrata*, exhibited decreased growth and reproductivity when maintained under crowded conditions in the laboratory. Berrie⁵ corroborated that study with observations of natural populations of *Biomphalaria* in Uganda. We studied the phenomenon in *Fossaria cubensis*, a snail vector of fascioliasis with some observations on *B. glabrata*. The *Fossaria cubensis* used in these studies were descendants of individuals collected in Angleton, Texas, where they are natural hosts to *Fasciola hepatica*. The snails were routinely maintained according to the methods of Isseroff and Read⁶ except that "Cerophyll" was used as food in place of hay infusion.

Each snail was maintained separately in 100 ml. of water in a 12.5 cm vessel. Each experimental or control group contained five to ten such snail samples. Unless otherwise noted, water was changed daily in all experiments; for controls distilled water containing a suspension of non-toxic chalk (as a calcium source) was used. In the experimental group water was replaced with fresh aliquots containing the same type of chalk but with the addition of the test factor(s). Temperature was maintained at 22° C.

To isolate the active substance, water from *Fossaria* cultures at least one month old, was evaporated at room temperature to about 1/100 of its original volume in large, shallow pans. The concentrated solution was dialysed against tap water and finally against distilled water, and the dialysed concentrate saturated with ammonium sulphate to precipitate the inhibitory substance. The precipitate was redissolved in a small volume of water and redialysed to remove the ammonium sulphate which can be toxic to the snails. Generally 1 ml. of the concentrated and purified inhibitory substance(s) (IS) was added back to 99 ml. of fresh water for assay purposes. On the basis of assays, dilutions for experimental purposes were adjusted according to a standard unitage defined as the volume of the concentrate required to just produce complete inhibition of growth of a 5.0 mg snail in 4 days. Fig. 1 shows the effect of the IS isolated with ammonium sulphate at unit concentration

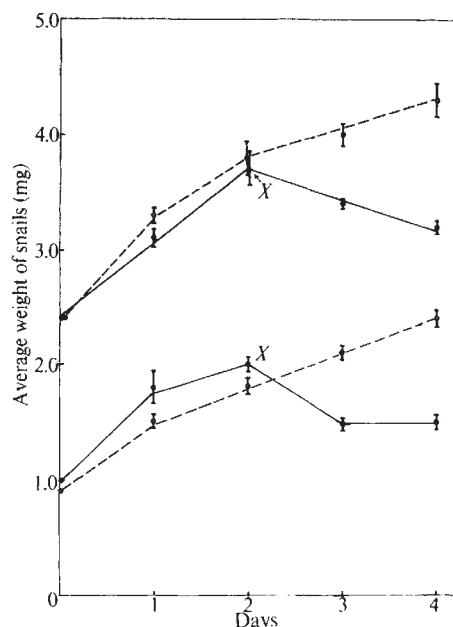


Fig. 1 Effects of unit concentration inhibitor on growth of *F. cubensis* in two age groups. Snails were permitted to grow for 2 days and IS was added after the second day to both groups (point ×). The untreated controls (○) continued to grow but growth inhibition and weight loss occurred within 24 h of addition of the inhibitor (●).

on *Fossaria* of two different ages. Growth is inhibited within 24 h after addition of the IS. A dose-response curve for IS shows its effect is non-linear with increasing concentration and has a maximum effect at about one unit of activity (Fig. 2). The IS effect was reversible. Growth resumed after exchange of IS water for fresh water on day 4, at a rate similar to that of untreated snails in the earlier part of the experiment. IS also affects egg laying by mature snails and this effect can also be reversed when the snails are placed in fresh water (Fig. 3). Eggs normally hatch in 9–10 days. In the presence of the IS development is normal till just before hatching, when the embryos die. It has, however, little or no effect on the growth of mature snails. An interesting cross species effect of the *Fossaria* IS on egg production of *Biomphalaria glabrata* was found; over a two week period egg laying of the schistosome carrying snail was inhibited by 56%. Specificity of the IS to molluscs is suggested by studies with planaria (*Dugesia tigrina*) and platyfish (*Xiphophorus*). At concentrations ten times those effective for *Fossaria* the IS showed no effect on growth of these organisms.

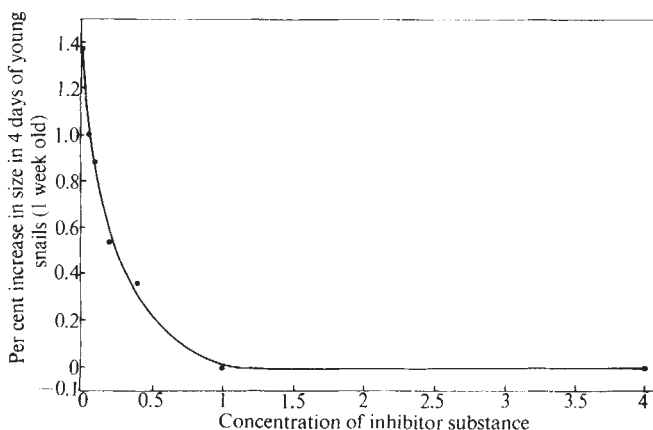


Fig. 2 Effect of increasing concentration of inhibitor on the growth of young *Fossaria*.

IS is stable for at least a month at 22° C in static, non-aerated, non-filtered tanks. At 5° C in concentrations of 1 to 1,000 it is stable for over 7 months, and is not affected by freezing, thawing, or lyophilization. Activity of the IS is completely destroyed by heating in solution at 85° C or by incubation with 1.0 ug of trypsin/ml. of diluted IS at room temperature (Fig. 4).

Our observations indicate that a chemical substance, presumably produced by *Fossaria cubensis*, can inhibit its own species growth and egg deposition. Although much work remains to be done with regard to completely isolating and purifying the IS, its physical and chemical properties indicate that it is probably a protein. Rose and Rose⁷ found that the growth inhibitor released by frog tadpoles is also proteinaceous. It would be interesting to determine whether the tadpole inhibitor and snail inhibitor produce their effect by a common mechanism.

The apparent absence of an effect on snail embryos until they are almost ready to hatch may possibly be explained by supposing that the gelatinous covering of the egg mass is impermeable to large molecules such as the IS until just before hatching when the covering breaks down.

It is likely that the production of IS under natural conditions may serve to restrict population increase during the dry season when food may not be abundant. The long stability of IS in solution at room temperature also supports this hypothesis.

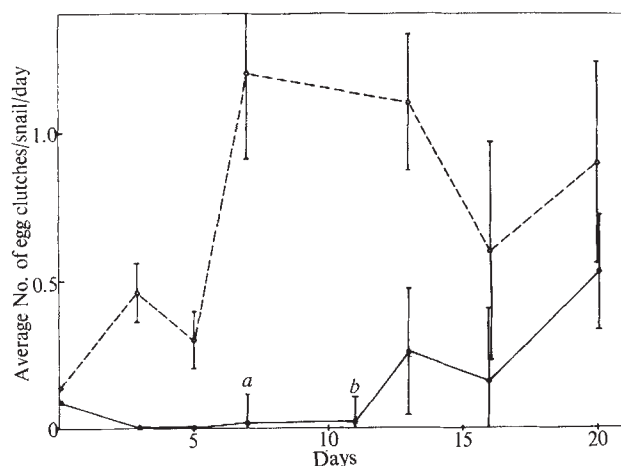


Fig. 3 Effect of unit concentration inhibitor on egg deposition in *Fossaria* (●). Egg laying (b) resumes when snails are placed in fresh water. (a) Upper curve (○) shows egg deposition during same period in fresh water.

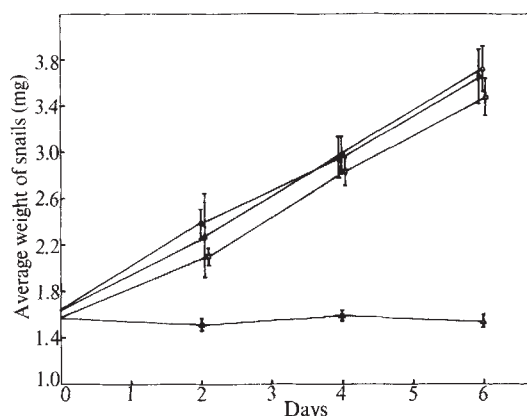


Fig. 4 Effect of adding trypsin (1 µg/ml.) to cultures of *Fossaria*. Controls containing fresh water only (□) or fresh water plus trypsin (○), were not statistically different in growth rate from snails grown with inhibitor (unit concentration) and trypsin (●). However, growth in the presence of inhibitor (unit concentration) without trypsin (△) was almost completely inhibited.

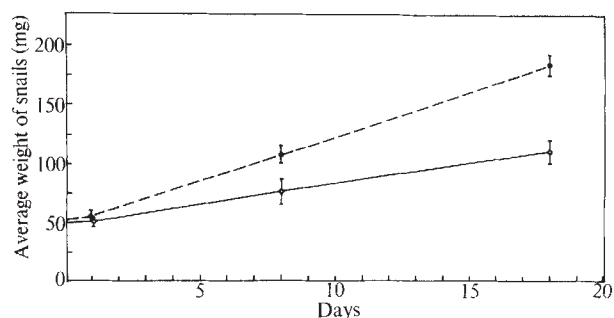


Fig. 5 Effect of unit concentration *Biomphalaria* IS on the growth of young *Biomphalaria*.

An ammonium sulphate precipitate prepared from old cultures of *Biomphalaria glabrata* has also been found to have an inhibitory effect on young *B. glabrata* (Fig. 5) and on egg production by the adults (Table 1). Experiments with *Biomphalaria* were carried out by methods similar to those used with *Fossaria*.

Table 1 Effect of Old Water from *Biomphalaria* Cultures, after Dialysis and Redilution to its Original Concentration, on Egg Laying in *Biomphalaria*

	Average No. of egg clutches/snail/day	Inhibition	Significance
Controls	15.2 ± 2.84	—	—
Experimental with old water	8.6 ± 1.12	43%	$P < 0.05$

Data taken from a 16 day experiment. Five snails in each group.

This work was supported in part by a grant from the Research Foundation of the State University of New York.

M. G. LEVY
M. TUNIS
H. ISSEROFF

Departments of Biology and Chemistry,
SUNY College at Buffalo,
Buffalo, New York 14222

Received February 7, 1972.

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Observation of the Pleurotomarid *Entemnotrochus adansoniana* in its Natural Habitat

THE pleurotomarids or slit-shells are the most primitive of existing gastropods and of unique malacological and conchological interest. They retain, in a particularly deep mantle cavity, what was probably the original molluscan organization of paired gills and associated organs¹. Externally they are large top shells but with a narrow slit running far back from the outer lip of the shell. The Pleurotomariacea were originally known only as fossils, appearing in the late Upper Cambrian and attaining the height of their development during the Palaeozoic².

It was therefore with an excitement similar to that which later greeted the discovery of the first living monoplacophoran, *Neopilina galathea*³, that in 1856 P. Fischer and



Fig. 1. *Entemnotrochus adansoniana* Crosse and Fischer, showing full extent of slit.

A. Bernardi described an empty slit-shell as *Pleurotomaria* (*Perotrochus*) *quoyana*. This had been found the previous year by Commandant Beau who collected in the Lesser Antilles and had taken it from a deeply laid fish trap into which it had been carried by its temporary occupant, a hermit crab.

This information is provided by Dance⁴ who adds, that, after passing through various hands, this famous shell became part of the Mrs de Burgh collection which was acquired by the British Museum (Natural History) in 1941. It is 4.52 cm in diameter and 3.42 cm high. More specimens have subsequently been taken off Barbados, Yucatan, and elsewhere in the Caribbean and the Gulf of Mexico. Descriptions of the shell features and the anatomy of this species were later given by Dall⁵ and Bouvier and Fischer⁶ respectively. A fuller account of the anatomy of the Japanese species *P. Beyrichii* was provided by Woodward⁷.

Fifteen species of *Pleurotomaria* divided into three sections on the basis of radular characters, *Entemnotrochus*, *Perotrochus* and *Mikadotrochus*⁸, are now known, seven from the West Indies⁹, the remainder from the Pacific, off Japan and round the Moluccas. All live in relatively deep water.

The first shell of *Entemnotrochus adansoniana* Crosse and Fischer, 1861, was, in the words of Dance⁴, "discovered in the collection of a Dr Commarmand, unlabelled and partially broken, and it was twenty years before complete specimens were found, in the Caribbean, by the US Coast Survey steamer Blake". This second Atlantic species is some three times larger than *P. quoyana*, the dimensions of a specimen now in my possession being 14.1 cm in diameter and 13 cm high, the slit being 16 cm long (Fig. 1). Among slit-shells, this species is only exceeded in size by the Pacific *E. rumphii*.

The specimen illustrated in *Rare Shells*⁴ has an interesting history, being one of a pair originating at Tobago and purchased at Barbados by an enthusiastic Victorian collector, Samuel Archer, an army surgeon from Liverpool¹⁰. One of the shells is now at the Liverpool, and the other at the Manchester Museum. Occasional specimens of this species have been taken during the past century, usually in depths of around 100 fathom, from the Caribbean south to Brazil.

It can now be stated to occur, and undoubtedly in some

numbers, at depths of around 400 foot downwards, on the sediment covered talus slopes off the seaward face of the coral reefs along the north coast of Jamaica. These observations were made from a two-man 'Nekton' submersible operating from the Discovery Bay Laboratory¹¹ during August 1972.

Following intensive study over many years by the late Professor T. F. Goreau and colleagues^{12,13}, the reefs in this vicinity are the best known in the Caribbean, and probably in the world. Shallow water and scuba diving studies have revealed a series of zones. Inshore areas involving a shallow lagoon and a reef crest are followed by an extensive fore-reef, in exposed areas involving buttresses, and characterized by massively branching *Acropora palmata* with often hemispherical colonies of such corals as *Monastrea annularis*, *Millepora alcicornis*, *Porites astreoides* and *Diploria strigosa* and below this an area of delicately branching, frequently broken, *Acropora cervicornis*. These occupy a gently shelving fore-reef which is succeeded by a steep fore-reef slope largely covered with sandy deposits with small species of the green calcareous weed, *Halimeda*. This comes to an abrupt end at around 150 foot where it gives place, at the "drop-off", to a vertical, in places actually overhanging, wall occasionally interrupted by shutes down which sediments periodically cascade. At the base of this wall, around 400 foot, the talus slope begins. This descends at an angle of about 45° into deep water, and appears to consist of fallen blocks and fragments covered with a thin layer of soft sediment.

The highly manoeuvrable 'Nekton' submersible (General Oceanographics, Inc.) proved an ideal vehicle for making biological and sedimentological observations from the lower level of Scuba to a depth of some 1,000 foot. The bottom could be viewed in close detail through the numerous well placed port holes in and around the nose (where the observer lies) and around the conning tower in which the pilot sits.

Epifaunal molluscs are singularly few in these environments but a large top-shell was seen on my first dive. Dr Willard Hartman reported seeing another in which he perceived indications of a slit and later a dead shell of *E. adansoniana* was secured by means of the submersible's "claw". On my last dive (piloted by Dr Land) a fine specimen was closely observed at a depth of 525 foot. It was alongside a projecting sheet-like expanse of grey sponge. The foot was partly expanded and the slit clearly lined with tissue but the epipodium with its tentacles was withdrawn. Although the entire surface of these upper talus slopes was covered with a silvery coloured layer of sediment, this was very thin and the animal must normally crawl on the hard surface immediately beneath this and is probably just as much restricted to a hard substrate as are the shallow-water Trochacea. Later information from Discovery Bay is of specimens seen on the rocky surface of the wall, as high as 380 foot, as well as one on the talus slope at 1,000 foot. Movement must be very slow because any significant disturbance of sediment would foul the deep mantle cavity with its elaborate equipment of two aspidobranch ctenidia (that is, four rows of filaments compared with the single row in the pectinibranch ctenidium of mesogastropods and neogastropods). All aspidobranch gastropods are endangered by water borne sediment¹.

The nutrition of so large an animal raises problems. The population of the escarpment above consists largely of sponges (including numerous colonies of Sclerospongiae¹⁴) feeding on fine phytoplankton and of gorgonids living on zooplankton. The only other conspicuous animals there and on the talus slopes are crinoids, ciliary feeders which may be collecting plankton mixed with fine organic debris. There is none of the algal life on which archaeogastropods, from *Halotis* to docoglossid limpets and trochids, characteristically feed by means of the broad rhipidiglossan radula.

As with other groups, including ascidians and bivalve

molluscs, descent into deep water has involved at any rate a partial change to a carnivorous diet in the pleurotomarids. Woodward found clear evidence that *P. Beyrichii* feeds on sponges and this is highly probable for *E. adansoniana*. However, there is the problem of the rhipidoglossate radula, here of unique width with 69 teeth on either side of the central tooth in *E. adansoniana*⁵, 109 in *P. quoyana*⁶ and 111 in *P. Beyrichii*⁷. These are of various merging types only two of which appear capable of tearing sponge tissue⁷; the function of the remainder remains obscure.

There is a possibility that these may be concerned with collection of fragments of weed, especially *Halimeda*, falling from above. The large *H. copiosa* Goreau and Graham is common between 30 and 240 foot especially on steep slopes¹⁵ and was observed in abundance round the top of the escarpment. The surrounding area was covered with whitened accumulations of the characteristic segments of dead fronds. These must continuously rain down on the upper regions of the talus slope where *E. adansoniana* lives and may retain significant amounts of edible matter. This upper talus slope could well represent a unique environment particularly rich in constantly renewed supplies of organic debris.

However, there is now a possibility of resolving this and other problems concerning the life of these slit-shells, notably the functioning of the pallial and reproductive organs with information about development.

I was also able to examine specimens of *Basilomya goreau* nov.gen.nov.sp. recently described by Bayer¹⁶, obtained by Dr J. B. C. Jackson (Johns Hopkins University). This small dimyid bivalve, which lives on the under surface of the extensive and very thin sheets of *Agaricia* which occur around the lowest level of coral growth, was first collected by Professor Goreau in 1964. This appears to be the first time any dimyid has been examined in life.

I thank Dr Lynton Land (University of Texas) for his invitation to participate in this North Jamaican Reef escarpment project financed by the National Science Foundation with essential logistic support from D. C. Tretzel and the Kaiser Bauxite Co. and the Royal Society for a personal grant.

C. M. YONGE

Department of Zoology, University of Edinburgh,
West Mains Road, Edinburgh EH9 3JT

Received September 13, 1972.

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Specificity and Inhibition of Attraction of Male *Rhyacionia frustrana* and *R. rigidana* to their Female Sex Pheromone

Rhyacionia spp. moths (Lepidoptera: Olethreutidae) are common pests of young pines in the Southeastern United

Table 1 Average Number of Male *Rhyacionia* spp. Moths Caught per Trap by Live *R. rigidana* Females, Crude Tip Extracts of *R. frustrana* and *R. rigidana* Females, and Combined Extracts*

	<i>R. frustrana</i> extract	<i>R. rigidana</i> extract	<i>R. rigidana</i> females	Combined extracts	Control
Male <i>R. frustrana</i>					
Date					
March 27	28	0†	0	NT	0
28	15	NT	0	0	0
29‡	0	0	0	0	0
30	23	0	0	0	0.3
April 1	NT	NT	0	0	0
2	8	NT	0	1	0
Total	74	0	0	1	0.3
Male <i>R. rigidana</i>					
Date					
March 27	0	59†	9	0	0
28	0	NT	57	0	0
29‡	0	0	0	0	0
30	0	30	39	0	0
April 1	NT	NT	10	0	0
2	0	NT	0	0	0
Total	0	89	115	0	0

NT, not tested.

* Each figure represents the average of one to three traps with 5 female equivalents of one species, combined 5 female equivalents of both species, or five live females per trap.

† Fourteen female equivalents.

‡ Temperature ranged from 10° C to 4.5° C. No flight activity of moths was observed.

States. Although the Nantucket pine tip moth, *R. frustrana* (Comstock) is usually the most prevalent species, it is often associated with the pitch pine tip moth, *R. rigidana* (Fernald)^{1,2}. No previous studies have provided information on mechanisms which isolate these two species.

During the 1972 spring emergence of *Rhyacionia* spp. moths, a study began in Clarke County, Georgia, to determine the role of sex pheromones in separating the two species. Approximately 2,000 infested shoots of loblolly pine, *Pinus taeda* L., were collected from two plantations and placed in a continuously illuminated screen cage (1.2 × 2.5 × 2 m) indoors. Moths were removed from the cage as they emerged and held in glass vials. The moths were separated by species using characters as previously described¹. Females were held in the vials for one day and then the apical two or three abdominal segments were clipped off and placed in hexane-ether (1 : 1).

When moths began to emerge in large numbers in the field in late March, traps were baited at dusk on several dates with five equivalents of tip extract of female *R. frustrana*, *R. rigidana*, or a combination of both extracts, or with five live female *R. rigidana*. No traps were baited with live *R. frustrana* because it had already been demonstrated that males are readily attracted to caged females^{3,4}. The trap design was the same as that described before⁵. Live females were placed in a cage in the trap and crude tip extracts were pipetted onto filter paper and placed in the trap. Unbaited controls contained filter paper only. The traps were placed in a single row about 2 m high in infested pines which averaged 2.5 m. All traps were oriented in the same direction and were spaced about 5 m apart. Traps were systematically placed so that no two identical treatments were adjacent.

The catches of male moths per trap are shown in Table 1. Crude extracts of *R. rigidana* females attracted only *R. rigidana* males and compared favourably with live females with an average of 4.9 males/female equivalent/day and 3.6 males/live female/day respectively. We have previously shown a similar relationship in *R. frustrana* emerging in the spring⁴. In the present study, crude *R. frustrana* extracts caught an average of 3.06 *R. frustrana* males/female equivalent/day.

On the basis of moths emerging in the indoor cage, *R. rigidana* made up about 10% of each of the two field populations. However, more *R. rigidana* males were trapped than *R. frustrana*

indicating a stronger response to the pheromone by the males or more pheromone per *R. rigidana* female.

Traps baited with a mixture of five female equivalents each of *R. frustrana* and *R. rigidana* extracts on five occasions caught only one *R. frustrana* male but on the same nights *R. frustrana* and *R. rigidana* extracts in separate traps caught an average of 15.3 and 35.3 males of their own species per trap respectively. No cross attraction occurred with females of either species; therefore, it seems that the pheromones are different and effectively aid in isolating the two species.

Table 2 Attraction of *Rhyacionia frustrana* Males to Different Quantities of Female Tip Extracts

No. of males trapped		
1 FE 1 ± 1	2 FE 5 ± 1	5 FE 15 ± 9

Each figure (± 1 s.d.) is the average number of males attracted to traps containing 1, 2, or 5 female equivalents (FE) per trap in each of eleven replications with one trap per replication.

In other lepidopteran species, inhibition of male attraction has been demonstrated with live females⁵ and with synthetic compounds⁶⁻¹⁰. In one interesting case with synthetic compounds¹¹ evidence was presented that the sex pheromone of one species of Gelichiid moth consisted of the *cis* isomer while that of a second species consisted of the *trans* isomer of the same compound. Mixtures (10 µg each) of the two isomers inhibited attraction of males of both species. Attraction or inhibition of attraction was not compared with live females or extracts of females; therefore, it is not known whether the sex pheromones of these two species are multi-component.

Wright's suggestion that an insect attractant may be composed of more than one chemical¹² has been supported recently with several species of moths. In most of the cases in which this point has been carefully examined, two or more components of the natural sex pheromone have been demonstrated or indicated^{7,13-18}. Our data indicate that there are two or more components of the sex pheromone system of *R. frustrana*. Field tests of gas-liquid chromatography (GLC) fractions of crude extract have shown that at least two fractions were attractive alone and more attractive when combined. Also, combined GLC fractions were considerably less attractive than crude extract. According to comparisons with GLC peaks containing attractant activity, the crude extracts of female tips (five female equivalents) which completely inhibited attraction of *R. rigidana* males, contained a maximum of about 20 ng of any one attractive component.

The amount of extract from *R. frustrana* females which completely inhibited attraction of *R. rigidana* males (five female equivalents) was quite close to the attraction threshold for *R. frustrana* males (approximately one female equivalent, Table 2). These data clearly emphasize the potent inhibitory properties of these extracts.

This report is the first demonstration of complete inhibition of male attraction with extracts of females containing the full complement⁴ of the natural sex pheromone system. In addition to the previously demonstrated opposite isomer type of inhibition of attraction¹¹, a different system of inhibition may be operating in cases involving multi-component sex pheromones.

C. WAYNE BERISFORD
U. EUGENE BRADY

Department of Entomology,
University of Georgia,
Athens, Georgia 30601

Received July 3; revised October 4, 1972.

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Chromosome Aberration Dosimetry in a Case of Over-exposure to Radiation

ESTIMATION of radiation dose to the body from the yield of dicentric chromosome aberrations in peripheral blood lymphocytes has been developed into a valuable technique in radiological protection practice¹. It has been used as a means of resolving disagreements arising from anomalous film badge readings, and in cases of possible over-exposure where no dosimeter was worn. Another use is in the accurate estimation of dose which is necessary for determining the future employment status of exposed radiation workers, and also for guiding clinicians in the treatment of radiation injuries.

In January 1970 a radiation worker was referred to hospital with lesions on both hands; he had been working for three years with iridium-192 sources which were being used for industrial radiography purposes. Radiation injury was diagnosed. Nevertheless he continued in radiographic work until December 1971 when he was admitted to hospital where the index finger of the right hand was amputated. No satisfactory prior dosimetric data were available and it was decided to use the chromosome aberration technique in order to make an assessment of accumulated radiation dose.

From a blood sample lymphocyte cultures were made and five hundred metaphase spreads were examined with the following results: dicentrics, 49; centric rings, 7; acentric rings, 10; interstitial deletions, 31; terminal deletions, 21. Eighteen dicentric chromosomes were also found minus their associated deletions. A detailed account of the genesis and significance of dicentric and other chromosome aberrations is given by Evans² and UNSCEAR³.

As about five years elapsed between the first exposure and the chromosome analysis it is necessary to take into account the rate of loss of lymphocytes from the body. By studying the fall of aberration yield with time after treatment in a group of radiotherapy patients, Norman *et al.*⁴ estimated the half-life of lymphocytes carrying dicentric aberrations to be approximately 530 days. Buckton *et al.*⁵ estimated 1,600 days with 95% confidence limits of 900 to 6,700 days. Our own findings of 30 months (in preparation) falls between these two estimates. By taking a round figure of 1,000 days as the half-life of the lymphocytes containing dicentric aberrations, and by assuming that the radiation was received uniformly in equal annual doses during the five years, it is possible to predict that 90 dicentrics would have been found in 500 cells if no cell losses had occurred.

By reference to a calibration curve produced *in vitro* (in preparation) using cobalt-60 gamma radiation at a chronic dose rate of 18 rad⁻¹, this dicentric yield corresponds to an

equivalent whole body exposure of 200 rad. As the person probably received the radiation dose in small increments at high dose rates spread out over five years, this might not be the appropriate calibration curve to use but we have not yet investigated the effects of dose fractionation.

We also observed in the 500 cells 18 dicentric lacking their associated fragments. However, because the calibration curves are based solely on dicentric yield in complete cells⁶ it is not possible to interpret them in terms of dose. The absence of the associated deletions suggests that mitosis may have occurred *in vivo* following irradiation. Such divisions are normally rare, but when they occur the fragments, which lack a centromere, pass wholly into one or other daughter cell. Bearing in mind that the average lifetime of mature lymphocytes may be several years, Bender⁷ states that the presence of cells lacking expected fragments may indicate that the chromosome damage has been sustained over a considerable period. This theory is in agreement with the present findings where the history of malpractice is known to span five years.

Studies of this nature, especially where there is a lack of reliable physical dosimetry data, illustrate the valuable contribution which chromosome analysis is making to good radiological protection practice. In addition, the accumulation of this type of data is necessary for the eventual evaluation of risk of the late effects of radiation in terms of acute biological effect.

We thank Mr C. D. Burgess of HM Factory Inspectorate and Miss E. J. Eltham for assistance.

D. C. LLOYD
R. J. PURROTT
G. W. DOLPHIN

National Radiological Protection Board,
Harwell,
Didcot, Berkshire

Received July 13, 1972.

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Neuromuscular Control of Trumpeters' Lips

SCIENTIFIC experiments on musical performance are surprisingly sparse when one considers that neuromuscular control of skilful motor performance reaches its acme in music. A case in point is the embouchure of brass instrumentalists, that is, the control of the firmness and vibration of the lips in relationship to each other and to the mouthpiece. The pedagogical literature is in a "state of chaos" according to Weast¹. Among anatomists little more is known today about the normal function and kinesiology of the muscles of facial expression than has been known for a century—knowledge gained through the dissection of corpses and simple observation². With the exception of buccinator muscle during normal function^{3,4} and labial muscles during speech^{5,6}, the facial

muscles have been almost ignored by electromyographers. Thus, electromyography (EMG) offers to reveal the function of the facial muscles as it has for many other parts of the body⁷.

With both science and art as our motives, we studied the effects of register, intensity and subjects' proficiency on the electromyographic potentials of selected facial muscles during trumpet performance by eighteen players with a wide range of experience. We recorded from bipolar fine-wire electrodes⁷ in the orbicularis oris muscle in both the upper and lower lips, levator anguli oris and depressor anguli oris. Our eighteen volunteer trumpet players possess a wide range of proficiency; at the top are three concert artists and at the bottom, three high school students with limited experience. The subjects were rank-ordered according to numbers of years of private study, years played and age. All subjects were exposed to the same controlled environment, technique, instrumentation and procedures. After a set warm-up period, each subject played on cue a series of fifty-one test items for recording; these were chosen to demand a wide variety of trumpet techniques, including extremes of dynamic and pitch range. EMG activity was quantified using a four-integer system as follows: 0, nil; 1, slight; 2, moderate; and 3, marked (maximum). When testing for differences between related scores, that is, when comparing two measures on the same subject, we used the Wilcoxon matched-pairs signed-ranks test. For comparisons between independent scores, that is, measurements made on two different groups of subjects, we used the Mann-Whitney U test, as recommended by Siegel⁸.

Both register and intensity positively affect the embouchure's muscle activity ($P < 0.005$), register having a greater effect than intensity ($P < 0.005$).

Advanced trumpeters have more activity in the muscles surrounding the lips than those in the lips ($P = 0.025$) in contrast to the reverse finding with less advanced trumpeters who show no difference. This negates the widely accepted "drawstring or tug-of-war" theory which proposes that the lip muscles are pitted against the other facial muscles in the correct embouchure formation. The beginners demonstrate more muscle activity in the upper lip than in the lower lip ($P < 0.005$). Although the lip muscles can be considered anatomically as a single unit (a sphincter muscle surrounding the oral orifice), functionally the lips must be considered as two separate muscles. While the data do not show that advanced trumpeters have less muscle activity in the upper lip than in the lower lip, advanced trumpeters do show a smaller ratio of upper lip to lower lip activity than beginners. This finding suggests that beginners should either concentrate more activity in the lower lip or less activity in the upper lip, or both; but the present data do not disclose which of these possibilities caused the difference between groups.

JOHN V. BASMAJIAN
ELMER R. WHITE

Regional Rehabilitation Research and
Training Center,
Emory University,
Atlanta, Georgia 30322

Received October 2, 1972.

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BOOK REVIEWS

Transparent Things

Foundations of Cyclopean Perception. By Bela Julesz. Pp. xiv+406. (University of Chicago: Chicago and London, 1971.) £9.

Foundations of Cyclopean Perception is an exciting, stimulating book about one of the most interesting recent developments in perceptual psychology. It is also beautifully produced, worth buying as an object in its own right. As a book it can be read and understood on several levels. It is a mixture of transparent clarity—most of the experiments the author discusses can be attempted by the reader—and of abstraction which can be difficult and even ambiguous.

Julesz has worked on binocular interactions in human vision for about 15 years. His fundamental technique is to use a pair of patterns which give a depth percept when fused binocularly, as in a stereoscope, but which contain no monocularly perceivable form. The advent of large digital computers made the generation of such stimuli possible: perhaps Julesz's greatest achievement is his combination of modern technology with conceptually simple experiments. A typical stimulus is a pair of patterns of equal numbers of black-and-white square picture elements arranged at random in 100 element square arrays, giving the impression of randomized chess boards. Both patterns of a pair are identical except that, say, a central square portion of the array in one is shifted horizontally by an integral number of picture elements with respect to the same region of the other. When the two patterns are fused binocularly the lateral disparity leads to the percept of a central square region standing out in depth from the remainder of the fused array, which forms a rectangular surround in a different depth. Although the disparities cannot be detected by visual inspection of the separate patterns of each pair, the retinal disparities between the fused images are perceived. In normal vision such differences in retinal disparity between regions of fused images automatically lead to the perception of depth, but before Julesz's work it was assumed that the disparity

information could not be used without prior recognition of forms in the visual stimulus. A clear conclusion from this kind of experiment is that depth perception, and the underlying processes required to detect the disparities between two retinal images, can be entirely central processes not requiring monocularly recognizable structure and independent of eye movements and vergence. Not only is it possible to draw conclusions about depth perception, but it is also possible to use Julesz's technique to distinguish the central and peripheral components of a wide range of visual phenomena. Many such experiments are described in the book, and the significance of their results to visual pattern processing and to general theories of neuronal function is discussed in detail.

Julesz's technique has led to two kinds of achievement; a much better understanding of the mechanisms of binocular vision and depth perception, and a method for locating function within the central nervous system. I shall not list his major results, but I do think that it is worth stressing their relevance to the most difficult unsolved problems of perceptual psychology. First, he shows clearly that "familiarity deprivation" can lead to novel and surprising insight. New kinds of stimulation may be necessary for new discoveries. Second, as had been well understood by many earlier workers, the use of binocular interaction is an extraordinarily powerful tool for locating visual function. Obviously it provides a way of separating peripheral from central components of a percept, but more than this, a hierarchy can be built up, in which the relative level of a function can be assigned a place. For example, in some circumstances it is clear that motion perception can occur after fusion, at a higher level than the pattern-matching process for random dot stereograms. In this case the stereograms were used to separate cues for movement that are inextricably mixed in normal vision.

There are several irritating aspects to the book—for example, considerable redundancy—but as a whole it is a huge

achievement, making an original synthesis of visual perception and providing stimulation and suggestions for experiment that will be hard to exhaust. I think that some of the material is included prematurely, for example, sections on eidetic imagery and model building, but for many readers these may well provide a feeling of more immediate contact with work actually in progress.

In short, Julesz has written an original, idiosyncratic, immensely exciting and beautiful book. It deserves a wide audience, particularly because almost all the experiments described can be performed by the reader using the analogy representations of Julesz patterns (which, incidentally, are of the highest quality that I have seen printed). The accessibility of the results makes it much easier to accept Julesz's slightly dogmatic style, and gives one a much greater feeling for the synthesis of perceptual psychology and neurophysiology towards which Julesz is continually pointing, and without which an understanding of perception is impossible.

ANTHONY ROBERTSON

Wallace and Darwin

Wallace and Natural Selection. By Lewis McKinney. Pp. xix+193. (Yale University: New Haven and London, September 1972.) \$12.50; £4.95.

DR MCKINNEY has given more than six years of close study to Alfred Russel Wallace. He has traced minutely the intellectual and empirical sources of that great naturalist's thoughts on selection, and has carefully documented the relations between Wallace and Darwin. The results appear in this book—an elegant and readable piece of scholarship, drawing together a vast quantity of published and archival material. The microscopic examinations of Wallace's deception over the actual place of his "sudden insight" into the mechanism of evolution, and the even more interesting deception by Darwin over Wallace's 1858 paper, are fine examples of historical detection. Some intriguing ques-

tions emerge, and we can perhaps expect McKinney to pursue these in future writings.

On broad historiographical grounds, however, there are two grave defects in this book. The first is McKinney's unconsidered approach to the way religion and science interacted in the nineteenth century. He cites the historian Hooykass ("religion→science") favourably, but elsewhere couches his descriptions of Wallace in terms that overstress his own ahistorical biases: Wallace was "essentially unprejudiced by religious preconceptions"; "unencumbered by religious ties", and so on. This language is too value-laden.

Secondly, McKinney seems to be wholly unaware of the social and political contexts of evolutionary biology. Some of the very material he cites patently indicates Wallace's early meritocratic view—a view which needed the philosophical (and psychological) support provided by natural selection theory. McKinney does not catch this.

Nor does he seem to know of recent work by R. M. Young and others on the specific ideological backgrounds of Chambers, Malthus, Darwin and Wallace himself. Natural selection was such a useful political tool, and arose at such an opportune time, that it is hard to believe its originators were unmotivated by "external" (that is, non-scientific) factors. The single external factor in McKinney's book (aside from religion, which he mishandles) is Wallace's ambition. Otherwise, McKinney concentrates only on intellectual motives, and though he does this very well, the resulting explanation is insufficient for modern history.

It is as if by reading *Vestiges*, Lyell and Malthus, spurning religion and dabbling in natural history (which is all Wallace did at first), anyone could have hit on a mechanism of evolution, perhaps natural selection itself. Yet hundreds of intelligent men did just these things (in spite of McKinney's denial—unfootnoted—of widespread atheism) without such results. So what really did happen that led Wallace to this grand theory?

As yet, Dr McKinney has provided only a fraction of the answer. Because he is such a fine scholar, however, we must hope that he can widen his view.

J. R. FRIDAY

Atmosphere of Stars

Basic Physics of Stellar Atmospheres. By T. L. Swihart. Pp. viii+86. (Pachart: Tucson, 1972.) \$7.95.

THIS is a short text covering the theory of stellar atmospheres for the non-specialist. It consists of four chapters

covering the equation of transfer and formal solutions thereof, the grey atmosphere, model atmospheres and line formation. An appendix summarizes physical and astronomical constants and presents a number of exercises.

The book seems inferior to other recently offered stellar atmosphere texts. The aspects of the field treated in any detail exclude any of the important recent developments. For example, while the Avrett-Krook temperature correction procedure is derived in some detail there is nowhere mentioned the linearization methods developed by Auer and Mihalas which will soon displace the above procedure in essentially all aspects of model building.

The author explains in the preface that his approach is basically physical as opposed to mathematical. By this he apparently means that he includes specifically the microscopic physics that go into the radiation transfer process. In so far as he carries that philosophy the derivations and discussions of the input physics are very clear and readable. Yet I am amazed at the almost complete absence of a discussion of continuous absorption processes. A novice reading this as an introduction to the field would be left with the impression that essentially the only relevant source of continuous opacity is that of the H^- ion. The possibility of neutral hydrogen as a source of opacity is mentioned in only two sentences in the entire book. The neutral hydrogen absorption cross-section is never presented and the idea of opacity discontinuities is completely missing. This is somewhat surprising in that perhaps the most important aspect of model stellar atmospheres is their ability to predict the continuous spectra of stars and how they depend on temperature and gravity.

The lack of discussion of the macroscopic aspects of the physics of stellar atmospheres is also disturbing. Very few concrete results are given. What are typical densities in the photospheres of B stars and of M stars? What are the electron donors across the H-R diagram? Where is H^- important? Neutral hydrogen opacity? Molecular opacity? In short we find the discussions to be rather sterile and far more mathematically oriented than implied in the author's preface.

The execution of this book also leaves something to be desired. Different faced typing is often slightly displaced below the rest of the text which is disconcerting. The only two illustrations are interchanged. The rather thick covers contribute almost half of the total thickness of the book.

This book is the first of a series of "Intermediate Short Texts in Astrophysics" to be published by the author and his colleague, A. G. Pacholczyk.

As with any "first", there are many lessons that can be learned here.

D. M. PETERSON

Mycology from India

Hyphomycetes: An Account of Indian Species, except Cercosporae. By G. V. Subramanian. Pp. xi+930. (Indian Council of Agricultural Research: New Delhi, 1971.) Rs.50.

PROFESSOR G. V. SUBRAMANIAN is an acknowledged expert on the Hyphomycetes and a book by him dealing with this difficult group is to be welcomed. On opening the book it is, however, something of a disappointment to find that, although the publication date is 1971, the monograph was prepared in 1961 and contains no references to publications later than 1962. In view of the attention given to this group in the last decade by mycologists all over the world, including the outstanding contributions made by Professor Subramanian himself and his students, it is to be hoped that the publishers will give him the opportunity of bringing it up to date in the near future.

The first part (some 180 pages) gives an informative account of the early work on Hyphomycetes, of their general structure, habitats and importance in industry and agriculture, and discusses heterocaryosis, classification and nomenclature. In view of the author's own critical studies the section on classification is of particular value. A clear account is given of attempts up to 1961 to formulate a more natural arrangement than Saccardo's system which, while useful as a means of identification, brings together obviously unlike organisms in the same genera. Professor Subramanian adopts a system based on those of Hughes and Tubaki but gives his own definition of spore types. Since during the last decade there have been a number of other discussions and criticisms of the Hughes/Tubaki suggestions it is particularly unfortunate that this book does not contain Professor Subramanian's own more recent modifications of the system he proposed in 1961. It does, however, offer a firm basis for appreciation of recent contributions. This general section concludes with a useful bibliography of work published before 1962.

The greater part (Part II) of the book is devoted to descriptions of all species of Hyphomycetes recorded in India up to 1961/2, amounting to over 500 species. Necessary additions to and revisions of this part to bring it up to date are likely to be relatively unimportant. Part II begins with a useful key to genera and at appropriate places in the text keys are given to Indian species of each genus. Full and clear

descriptions of all genera and species are given, together with brief notes on habitat and distribution. Most species are illustrated by clear line diagrams.

This book represents years of painstaking and accurate work on this difficult group of fungi. In spite of the delay in its publication, it is a substantial and welcome addition to the literature and will prove to be invaluable, not only to Indian mycologists, but to all students of the Hyphomycetes and to plant pathologists.

The book is worthy of stronger binding (which does not stand up to normal handling of a volume of more than 900 pages) and of better quality paper. It is to be hoped that its success will warrant an early revision giving full justice to the author's scholarship and critical powers. L. E. HAWKER

Membranes and Ions

Biophysics and Physiology of Excitable Membranes. Edited by William J. Adelman, jun. Pp. xiv+527. (Van Nostrand Reinhold: New York and London, January 1972.) £12.25.

Membranes. Edited by George Eisenman. Vol. 1. *Macroscopic Systems and Models.* Pp. xix+333. (Marcel Dekker: New York, May 1972.) \$19.50.

It is curious that at a time when research on cell membranes has become such a fashionable field, there should be so few textbooks dealing adequately with certain aspects of the subject. The appearance of a book from which, to quote its dust cover, one "can gain a broad view of the theoretical approaches and technical procedures now used in excitable membrane biophysics" is therefore welcome. But as is too often the case with multi-author texts, the quality of the articles is variable and there are some surprising gaps in their coverage. Thus as far as voltage clamp studies are concerned, it seems a pity that a critical and helpful account of the application of the technique to myelinated fibres should be accompanied by one on giant axons that is marred by its cursory dismissal of the problems arising from the potential drop across the series resistance R_s due to the presence of the Schwann cell. As K. S. Cole points out in his historical survey, Hodgkin and Huxley took some pains to compensate electronically for at least part of R_s , but many of their successors have simply ignored the series resistance. In a fresh axon the resulting current-voltage curves may be displaced by many millivolts from their correct position. The subject is not well served by glancing over this admittedly technically troublesome point when at the same time consider-

able space is devoted to the questionable advantages of voltage-clamping with continuously varying command potentials instead of discrete steps.

It is surely odd that a chapter entitled "The Thermodynamic Foundations of Membrane Physiology" should make no mention at all of experimental investigations on the calorimetry of the nerve impulse. And although the mechanism of active transport of ions in nerve and muscle is dealt with very adequately, almost nothing is said about the measurement of the downhill ionic movements during nervous activity. There are useful chapters on other topics such as the X-ray analysis of myelin structure, optical studies, perfusion of giant axons, drug action, and experiments on model membranes. But the claim of the book to conduct a thorough "in-depth" examination does not seem entirely justified.

A similar claim is made for the volume edited by Professor Eisenman, this time more justifiably since it is the first of a series of unspecified length. Volume 1 sets the stage, containing accounts of the physical chemistry and theoretical background for the penetration of ions across different types of membrane, such as those with neutral pores, ion-exchange resin membranes, liquid membranes and dry silicate membranes. Volume 2 will be wholly concerned with the ionic conductance of artificial lipid bilayers incorporating carrier-type and tunnel-forming ionophores. This seems a logical scheme, and the last two chapters in Volume 1 are therefore a little out of place, consisting as they do of some purely theoretical arguments about excitable membranes, and of a mainly technical account of a new method for turning squid axons inside-out. In spite of the ingenuity of this procedure it is not clear that it has any marked advantage over existing perfusion techniques.

R. D. KEYNES

Human Error

Human Factors in Highway Traffic Safety Research. Edited by T. W. Forbes. Pp. xix+419. (Wiley: New York and London, June 1972.) £7.85.

SCIENTIFIC investigations towards an understanding of driving behaviour and a theory of accident causation have been conducted in such diverse fields as highway and traffic engineering, ergonomics, psychology, and driver education. Perhaps because of this, such research is not always accessible to the engineers who are charged with the task of designing a safe transport system for human use. Human factors is one of the newer engineering disciplines which attempts to assess the

design implications of behavioural studies.

This book, which has contributions from seventeen authors, specialists in a particular area of human factors studies, summarizes research on the biographical and physiological characteristics of drivers, factors in sign legibility, other methods of presenting information to drivers, skills, judgment and information acquisition in controlling vehicles in traffic, impairment of driver performance due to such factors as fatigue, alcohol and drugs, the effect of psycho-social factors such as stress, driver education and improvement. It can be seen from the comprehensive range of topics covered that of necessity it can only serve as an introduction. Each section is extensively referenced, however, thereby making a very valuable contribution. Consequently this may be of interest to students on courses of highway and traffic engineering as well as practitioners in the field of road safety. The extent to which it can be effective in arousing students' interest in the implications of their work as engineers, as an isolated course, is unknown.

The main area of concern, however, is the extent to which an approach such as this can achieve the stated objective of designing the man-machine environment task and/or system to fit human capabilities wherever possible. In principle this seems to be a very interesting approach. But most of the analysis is conducted in a vacuum quite distinct from actual accident occurrence. No attempts were made to show how this type of formal model, especially with regard to car following, overtaking, and so on, had any predictive validity. Indeed, the author concluded that drivers do a remarkably good job even in a system where breakdown appears to be inevitable. Perhaps this very observation suggests that the system analysis approach is not valid.

The cybernetic models proposed in most chapters to describe the driver-car-road environment system equated man with a limited capacity information-processing channel and driving as a negative feedback control system. It seems unlikely that a theory of driving which makes such simplistic assumptions about the driver will be very useful. Such a theory denies the possibility that he may initiate any action. Driving is only one of a range of social activities which is influenced by the behaviour of other people and as such is affected by the prevailing social mores which may be antipathetic to safety. While this book gives a good coverage of topics related to human factors, contributions from social psychologists are omitted.

Given this uncertainty about the validity of the model, the ability of this

type of approach to help the engineer design a system to cater for human capabilities may, therefore, be very limited.

JEAN SHAOUL

Biological Electron Spin

Biological Applications of Electron Spin Resonance. Edited by Harold M. Swartz, James R. Bolton and Donald C. Borg. Pp. x+569. (Wiley Interscience: New York and London, September 1972.) £11.55.

ELECTRON spin resonance spectroscopy was discovered in 1945, and first applied to biology in 1954. In 1958 Ingram produced the first book on applications of e.s.r. outside physics, and included a short chapter on biological and medical applications. The length of the present book shows that the subject is still growing according to an exponential law. How much has been accomplished in the 18 years since the first publication?

A quick glance through this book shows that e.s.r. is now being applied throughout biology, at the molecular level. It is particularly valuable in the study of photosynthesis; of enzymic reactions, especially those concerned with electron transport; of radiation biology and of various aspects of cancer. In the spin label method, which first appeared in 1965, there is a technique whose possibilities seem to be limited only by the ingenuity of the investigator. On the technical side of e.s.r. there have been great advances. Spectrometers are now reliable and easy to use, and the recent coupling of spectrometers to computers has led to improved spectra and improved analysis of spectra. The present book contains a detailed and valuable chapter on quantitative e.s.r. However, the basic problem remains, that on the one hand cells are complex and on the other hand e.s.r. spectra obtained from them are, in most cases, relatively nonspecific. So the use of e.s.r. has not led to any major advances in biology; nevertheless all biologists who are interested in the molecular aspects of their subject should be aware of the potentialities of the method.

How suitable is the present book as an introduction to e.s.r. for biologists and physicians? The editors, two physicians and a chemist, produced it because of their belief that the full potential of e.s.r. in biology was not being realized, principally because biologists did not have an adequate understanding of this method, and that a principal obstacle was the lack of a good text directed towards biologists. Accordingly the book begins with an explanation of the method, on a three-level approach, excessively simple in the introduction, and as simple as possible in the first three chapters, with more advanced material set off in small type.

Although all biologists and physicians will be able to follow the introduction. I am less sure about the explanatory chapters, where one is rapidly plunged into the mysteries of tensors, axes of symmetry, slow exchange and fast exchange, crystal field splitting etc. Most biologists and physicians, I feel, will heave a sigh of relief when they reach the more familiar ground of "Cells and Tissues" in chapter 4. My advice to them would be to skip the early chapters, to go straight to the applications, which are covered very thoroughly, and then, if they want to use e.s.r., to get the collaboration of a physicist or chemist who understands the technique.

The book is written by ten authors, and suffers inevitably from some lack of cohesion and some variability of standard. However, the treatment never drops below an adequate standard, and I recommend it as essential reading for all biologists approaching e.s.r. for the first time, and an essential acquisition for libraries.

S. J. WYARD

Nemerteans

Nemerteans. By Ray Gibson. Pp. 224. (Hutchinson: London, August 1972.) £3 hardback; £1.75 paperback.

THE nemerteans are an interesting but relatively neglected group and this new book is a timely and welcome addition to the well known Hutchinson University Library Series. Much of the book is a straightforward account of what is known about the structure and biology of nemertines. In this it will prove most useful to students of invertebrates and to marine biologists and also direct attention to subjects of interest which require further study. I would have preferred structure and function to have been associated more closely than they have been. The section on the mechanics of the body wall, body shape, locomotion and hydrostatic skeleton, for example, could with advantage have been included with the descriptions of morphology. Similarly, discussion of what little is known about osmoregulation might well have been included with the account of the excretory organs, the excretory system, excretion and the origin of the freshwater and terrestrial forms which comes elsewhere. In general this divorce of structure and function is what I disliked most about this book, which does contain, nevertheless, a wealth of clearly presented information. The contents of the chapter on general physiology and many of the facts presented in the chapter on ecology should, in my view, have been included with the descriptions of structure. Although the rise in the quality of production in this series has been accompanied by a concomitant rise in price, how much better

this new volume is in appearance as compared with the earliest issues in the series. Dr Gibson's drawings are excellent. There is a plate showing ultra-structural details and a colour plate illustrating *Geonemertes* (taken by Dr Batham) and the vascular system of *Malacobdella* visualized by the useful exopeptidase method done by the author.

This book will serve a useful purpose in stimulating further work on these interesting animals.

R. P. DALES

Dating Man

Calibration of Hominoid Evolution: Recent Advances in Isotopic and Other Dating Methods Applicable to the Origin of Man. (Proceedings of the Symposium held at Burg Wartenstein, Austria, July 1971.) Edited by W. W. Bishop and J. A. Miller. Assisted by Sonia Cole. Pp. viii+487. (Scottish Academic Press: Edinburgh; University of Toronto: Toronto, June 1972.) (Distributed in the UK by Chatto and Windus Ltd, published for the Wenner Gren Foundation for Anthropological Research, New York.) £7.60.

THE conference of which this volume is the product was concerned with recent advances in isotopic and other dating methods and their application to dating the emergence of man. The dating technologists and geologists included Thurber, Broecker and Bender, Bandy, Miller, Fitch, Cox, Dalrymple, Fleischer and Hart, Turekian and Bada, Bishop, Van Couvering, Brown, Curtis and Hay. The palaeontologists and archaeologists were Kurtén, Walker, Cooke and Maglio, Clark Howell, Pilbeam and Isaac. Nineteen chapters are followed by discussions, commentaries and three appendices.

This book is an admirable and very topical source book for all palaeoanthropologists and archaeologists. It contains the latest dates together with new interpretations of the evidence for man's evolution. The papers are all authoritative and the whole publication amounts to an invaluable reference work. One or two well-known names which might have been included are missing, but this hardly detracts from the value of the book. The interpretation of these new dates for man's evolution has very far-reaching implications, for reliable dating is an essential basis for any logical interpretation of the fossil evidence. It will be an invaluable reference work for all who are concerned with this rapidly-growing field of study, and will allow us all to reassess our ideas about man's origin and prehistory with considerably more confidence in the hardening data of Plio-Pleistocene chronology than we have been able to muster in the past.

BERNARD CAMPBELL

Solid Optics

Optical Properties of Solids. Edited by F. Abeles. Pp. 1026. (North-Holland: Amsterdam and London, 1972.) Dfl. 200; \$62.50.

THIS book is described on its inside cover as giving "an up-to-date account of the most recent advances in the field (optical properties of solids) . . . suitable both for graduate students and for those engaged in more advanced research". It contains eleven chapters written by various authors who have made substantial original contributions to the fields they review. Most of the articles were completed in early or mid-1968 and, in the four or five years since then, there have been many important developments which research workers cannot afford to ignore. The book will thus be primarily used as a source book for graduate and final year undergraduate courses on the optical properties of solids and perhaps also as an introduction to the field for new research workers. The editor points out in the preface that any textbook on solid state theory contains at least a few paragraphs devoted to optical properties. Sadly, most of the solid state physics texts used for final year undergraduate courses rarely do more than this and usually give virtually no account of the intrinsic optical properties of solids and the use of optical spectroscopy as a powerful tool to probe the electronic structure of both pure materials and crystal defects. This situation should improve as more books like this one become available.

The first chapter of this book gives a short review of some of the approximations used in the analysis of the optical properties of solids and this is followed by a good basic treatment of the intrinsic optical properties of semiconductors which, unfortunately, includes only three references to papers published later than 1967 and thus does not cover most of the advances made in this field during the last five years. Chapter 3, written by the editor, gives a valuable critical review of the optical properties of metals, including alloys, liquid metals and superconductors, and is much more up-to-date with even a few references to work published in 1971. This is followed by a chapter on modulated reflectance by B. O. Seraphin, a pioneer in this field, which contains a perceptive conceptual account of the relationship between intrinsic optical spectra and calculations of the electronic structure of solids. It includes a survey of the most important modulation techniques and their potential contributions to band structure analysis.

Chapters 5 and 6 give short accounts of the optical properties of non-crystalline solids and excitons respectively while chapter 7 describes magneto-optical properties of solids, principally

metals and semiconductors. This is followed by a chapter on photon-photon interactions in solids which has sections on multiphonon optical transitions, anharmonic effects, alloys and the effects of impurities (but with no discussion of laser Raman spectroscopy). Chapter 9 gives a balanced review of the optical properties of colour centres in alkali halides to 1968 with 400 to 500 references. There is no discussion of related topics such as the production of colour centres by radiation and, sadly, no reference to the many interesting developments that were described at the Reading conference in 1971. This is followed by a chapter on photoemission which describes the experimental techniques needed to obtain reliable spectra and surveys experimental results on metals and semiconductors. The final chapter, on second order optical processes in solids, covers the theoretical and experimental aspects of non-linear effects such as second harmonic generation, optical mixing and so on and, finally, a section on the theory of dipole susceptibilities in perfect crystals.

In a comprehensive text of this kind one would have expected to find chapters on the intrinsic optical properties of ionic crystals, and of crystals with layer structures, the use of isotropic and anisotropic stress and, particularly, the use of synchrotron light sources to obtain information on electronic transitions involving core levels and energy states away from the forbidden gap. The chapter on excitons could well have included at least some experimental data on exciton spectra and a discussion of topics such as exciton-phonon complexes and the effects of external perturbations on exciton spectra. The rather isolated chapter on colour centres might have been accompanied by a chapter on the optical spectra of impurity ions in solids. It is sad that an expensive and comprehensive book of this kind could not be more up-to-date or, at least, that other authors did not append notes on developments to 1971 of the kind that were provided for the chapters on magneto-optical properties and photoemission. There is now a clear need for a new volume on the optical properties of solids which will also cover the progress made since 1968 and in which the principal experimental techniques (modulation, synchrotron, magneto-optic, pressure, photoemission spectroscopy and so on) are all described but in which the information obtained by all these techniques on the electronic structure of each of the different classes of materials is also described together in detail. This volume will be an authoritative and well-written source book for this future work and, in the meantime, should be a useful addition to most science libraries.

M. R. TUBBS

Polymer Structure

X-Ray Diffraction by Polymers. By M. Kakudo and N. Kasai. Pp. xii + 464. (Kodansha: Tokyo; Elsevier: Amsterdam, London and New York, 1972.) Dfl. 125; \$39.

THE growing role of synthetic polymers in present day technology, and the realization that their useful properties depend largely on their semi-ordered molecular texture, is one of the factors that have led to the publication of books dealing specifically with the complex problems involved in the study of this texture by X-ray diffraction methods.

Another equally effective incentive is provided by the spectacular contributions of X-ray diffraction methods to our understanding of the structures of the natural polymers on which life is based; for these too are often semi-ordered. The highly ordered arrangements of molecules in crystals, and in the limited crystalline regions in polymers, are accessible by the well established methods to be found in textbooks of X-ray crystallography; but to deal in a quantitative way with the range of partially ordered textures from distorted crystalline to entirely amorphous (often in one and the same specimen), considerable extension of the mathematical treatment is necessary.

This latest contribution in this field is a translation of a recent Japanese textbook. It covers most aspects of the subject thoroughly and would provide an investigator with an adequate theoretical background for tackling the whole range of problems likely to be encountered. But it naturally has its own viewpoint which controls the treatment and the space allotted to different aspects; the emphasis is on the physics of X-ray diffraction rather than on polymer structure. It contains less information on the results of X-ray studies of molecular texture than other books of similar scope and intention published in the last few years; examples are naturally used in the exposition, but are treated only in so far as is necessary to display the methods. There is very little about the stereochemistry of polymer molecules or the relation between the properties of polymer specimens and such features as the proportion of crystalline material or the orientation of crystalline regions. This attitude is fair enough in view of the book's title, but inevitably makes it less interesting to polymer people than other books which devote more space to examples and the bearing of results on our understanding of polymer properties.

The book is divided into three sections: fundamental, experimental and analytical. It contains in its fundamental section more about the properties of X-rays than its rivals, and the development of the theory of diffraction

is more complete, starting from the scattering by a single electron and proceeding to atom, to molecule and thence to assemblies of molecules. This strictly formal approach permeates the whole book, which is an impressive achievement from the mathematical physicist's point of view. The style may appeal less to some investigators than the broader approach to be found elsewhere. The diagrams, so important in this subject which deals with three-dimensional structures, are on the whole good, though some are not as clear as they might be because the elementary trick of breaking a line where it passes behind another line has not always been used. The X-ray diffraction photographs suffer by being printed on the same paper as the text; some are very poor.

The analysis of the structure of well organized crystalline regions receives little attention; although this makes the book less complete than its title implies, the omission is justified on the ground that this aspect is, on the whole, standard X-ray crystallography which is well covered in other books.

This book would be the choice, if they can afford it, of those who like a formal academic approach to the problems of semi-ordered structures.

C. W. BUNN

Laser Beams in Plasma

Laser Interaction and Related Plasma Phenomena. Edited by Helmut J. Schwarz and Heinrich Hora. Volume 2. Pp. xiv+583. (Plenum: New York and London, 1972.) \$32.

INTENSE interest in laser fusion has been aroused recently by the declassification of some American work on the subject (*Nature*, **239**, 129; 1972). This work showed theoretically the feasibility of using laser beams to heat and compress matter to temperatures of the order 10^8 K and densities ten thousand times that of the initial solid state.

This book, dealing as it does with many of the key questions relating to laser fusion, is thus published at a very opportune time and is likely to be a best-seller. It is essentially the edited proceedings of the "Second Workshop" on the subject of laser interactions held at Rensselaer, Connecticut, in September 1971. There are thirty-eight research papers and some account of the apparently very abbreviated discussions which took place. Most of the material in these papers is now in the published literature, but it is nevertheless extremely useful to have it in a single book. Naturally the papers vary in standard and importance and the book would have benefited if some attempt had been made to show how the various aspects fit together in a summary article.

The papers are grouped in sections; in the first section on high intensity lasers there are two good papers by Beaulieu and de Maria on CO_2 lasers. But the rapid rate of progress in this field shows up here—there is, for example, no discussion of the new technique of electron beam excitation. There are half a dozen papers in the section on laser-induced breakdown, and the reader will no doubt be intrigued to discover that there is still no definitive answer to the question, "Where does the first electron come from?" In the section on diagnostics Kronast gives a good account of light-scattering experiments including a description of ways in which some more recent high resolution experiments show departures from the accepted theory.

Five papers make up the section on the interaction of laser plasmas with gases and magnetic fields. Probably the most fascinating is the account by Stamper of the spontaneous generation of magnetic fields in laser produced plasmas. A further five papers comprise the section which gives the book its title; that is, they deal with the interaction of laser light with plasma. This is possibly the most critical part of the laser fusion proposal because there is the danger that in the absence of non-classical processes the major fraction of the incoming laser light will simply be reflected. It is noteworthy that all the papers in this section are theoretical. Many of the possible non-linear absorption mechanisms are discussed here, although once again this is a subject on which new papers are appearing every week. Finally there is a section on fusion neutrons from laser-produced plasmas. This contains accounts of the much-publicized experiments in France, USSR, USA, Japan and Germany in which fusion neutrons have been detected.

In summary this book can give the near-specialist reader a very good feel for the state of the art in this subject as of late 1971.

R. J. BICKERTON

Information Diffusion

Invisible Colleges: Diffusion of Knowledge in Scientific Communities. By Diana Crane. Pp. x+213. (University of Chicago: London and Chicago, 1972.) £4.05.

ALTHOUGH half a dozen generations have passed since it was postulated that the proper study of mankind was man, the history of science increasingly supports the postulate. Darwin placed man firmly in the animal kingdom; Freud penetrated the recesses of his motivation; tomorrow we may spell out his genius in terms of atoms and molecules. But it is only in our genera-

tion that it was realized that man might not only look at himself through the eyes of science but that he might also look in the same way at science itself.

For two centuries science and society have interacted to give us technological man but only as we seem to approach the eleventh hour are questions being asked about the *modus operandi* of scientists. These two centuries saw science evolve from being the hobby of a few score gifted amateurs into a force subvented at a cost running into thousands of millions of pounds. Many questions have been asked about this activity since Bernal and Crowther concerned themselves with the social function and relations of science.

In her book *Invisible Colleges* Professor Diana Crane, a sociologist at Johns Hopkins, has addressed herself to one of them: how is scientific knowledge generated, and how does it diffuse? The question may seem naïve but her sociometric analysis shows that an answer to the apparently simple cannot be found at the drop of a hat.

Professor Crane selected two disciplines, mathematics and rural sociology, for her studies. This may seem idiosyncratic but lacking evidence from harder sciences—say, nuclear physics or molecular biology—there is no reason to question extrapolation to these areas. Her deduction is that the social organism that promotes the expansion of knowledge is, what she calls, "the invisible college", a coterie of researchers in contact though not necessarily working together, who exercise a measure of control on the expansion of knowledge in their speciality.

The growth of science has been deemed to follow a logistic curve: a slow start, exponential expansion followed by linearity, and then, as the new knowledge is absorbed into the body of science as a whole, a final stage of stagnation or even decline. Translated into human terms this means that a small but exciting nexus sets the pace, new blood is attracted and the field is set for exponential growth both in participants and publications. Extensive and intensive activity reaps its harvest and ultimately gives way to another up-and-coming region of endeavour.

We still have a long way to go before we fully understand the social institutions that produce ideas, be they in science or the arts. Professor Crane's significant and quantitative contribution to the subject reveals the ramifications of one—her invisible colleges. Readers in a hurry will appreciate the summaries at the end of most chapters which present the wood rather than the trees, but the quick synoptic vision will only partially sweeten the pill of having to pay more than £4 for somewhat less than 150 pages of text material.

ARCHIE CLOW